(54) Title: METHOD OF DIRECTING BIOSYNTHESIS OF SPECIFIC POLYKETIDES

(57) Abstract

A method to produce novel polyketide structures by designing and introducing specified changes in the DNA governing
the synthesis of the polyketide is disclosed. The biosynthesis of polyketide analogs is accomplished by genetic manipula-
tion of a polyketide-producing microorganism by isolating a polyketide biosynthetic gene-containing DNA sequence, identifying
enzymatic activities associated within the DNA sequence, introducing one or more specified changes into the DNA sequence
which codes for one of the enzymatic activities which results in an altered DNA sequence, introducing the altered DNA sequence
into the polyketide-producing microorganism to replace the original sequence, growing a culture of the altered microorganism un-
der conditions suitable for the formation of the specific polyketide analog, and isolating the specific polyketide analog from the
culture. The method is most useful when the segment of the chromosome modified is involved in an enzymatic activity associated
with polyketide biosynthesis, particularly for manipulating polyketide synthase genes from *Saccharopolyspora* or *Streptomyces*.

METHOD OF DIRECTING BIOSYNTHESIS OF SPECIFIC POLYKETIDES

Field of the Invention.

5   The present invention relates to a method for directing the biosynthesis of specific polyketide analogs by genetic manipulation. In particuar, polyketide biosynthetic genes are manipulated to produce precise, novel polyketides of predicted structure.

10   Background of the Invention
        Polyketides are a large class of natural products that includes many important antibiotics and immunosuppressants such as erythromycins, tetracyclines, and rapamycins. Their synthesis proceeds by an ordered condensation of acyl esters to generate carbon chains of varying length and
15   substitution pattern that are later converted to mature polyketides. This process has long been recognized as resembling fatty acid synthesis, but with important differences. Unlike a fatty acid synthase, a typical polyketide synthase is programmed to make many choices during carbon chain assembly: For example, the choice of "starter" and "extender" units,
20   which are often selected from acetate, propionate or butyrate residues in a defined sequence. The choice of using a full cycle of reduction-dehydration-reduction after some condensation steps, omitting it completely, or using one of two incomplete cycles (reduction alone or reduction followed by dehydration), which determines the pattern of keto
25   or hydroxyl groups and the degree of saturation at different points in the chain is additionally programed. Finally the choice of stereochemistry for the substituents at many of the carbon atoms is programmed by the polyketide synthase.
        Because of the commercial significance of *Streptomyces*, a great
30   amount of effort has been expended in the study of *Streptomyces* genetics. Consequently much is known about *Streptomyces* and several cloning vectors exist for transformations of the organism.
        Although many polyketides have been identified, there remains the need to obtain novel polyketide structures with enhanced properties.
35   Current methods of obtaining such molecules include screening of natural isolates and chemical modification of existing polyketides, both of which are costly and time consuming. Current screening methods are based on gross properties of the molecule, i.e. antibacterial, antifungal

activity, etc., and both *a priori* knowledge of the structure of the molecules
obtained or predetermination of enhanced properties are virtually
impossible. Chemical modification of preexisting structures has been
successfully employed, but it still suffers from practical limitations to the

5   type of compounds obtainable, largely connected to the poor yield of
multistep syntheses and available chemistry to effect modifications. The
following modifications are extremely difficult or inefficient at the present
time: change of the stereochemistry of the side chains in the completed
polyketide; change of the length of the polyketide by removal or addition

10   of carbon units from the interior of the acyl chain; and dehydroxylation at
unique positions in the acyl chain. Accordingly, there exists the need to
obtain molecules wherein such changes can be specified and performed
and would represent an improvement in the technology to produce
altered polyketide molecules with predicted structure.

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Summary of the Invention

The present invention provides a method to produce novel
structures from designing and introducing specified changes in the DNA
governing the synthesis of the polyketide. According to the method of the

20   present invention, the biosynthesis of specific polyketide analogs is
accomplished by genetic manipulation of a polyketide-producing
microorganism comprising the steps of:

(1) isolating a polyketide biosynthetic gene-containing DNA

25   sequence;
(2) identifying enzymatic activities associated within said DNA
sequence;
(3) introducing one or more specified changes into said DNA
sequence which codes for one of said enzymatic activities which results in

30   an altered DNA sequence;
(4) introducing said altered DNA sequence into the polyketide-
producing microorganism to replace the original sequence;
(5) growing a culture of the altered microorganism under
conditions suitable for the formation of the specific polyketide analog; and

35   (6) isolating said specific polyketide analog from the culture.
The present method is most useful when the segment of the
chromosome modified is involved in an enzymatic activity associated
with polyketide biosynthesis. The present invention is especially useful

in manipulating polyketide biosynthetic genes from *Streptomyces*, an organism which provides over one-half of the clinically useful antibiotics.

Brief Description of the Drawings

     FIG. 1 illustrates the organization of gene encoding polyketide synthase and designated *eryA* as follows: (a) Map coordinates of the DNA; (b) DOTPLOT of the output of COMPARE (window = 50, stringency = 32) program (Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin, Biotechnology Center, 1710 University Avenue, Madison, Wisconsin 53705 of *eryA* segment (x-axis) vs. subsegment of *eryA* comprises between 23 - 27.5 sequence coordinates (y-axis) [see Fig. 2]; (c) Open reading frame organization of *eryA* and enzymatic activities encoded. PT = propionyltransferase; ACP = acyl carrier protein; KS=β-ketoacyl ACP synthase; RmT = (2R) methylmalonyl CoA transferase; KR =β-ketoreductase; SmT = (2S) methylmalonyl CoA transferase; DH = dehydratase; ER = enoylreductase; TE = thioesterase; and (d) Schematic diagram showing the extent of each of the six modules in *eryA*.

     FIG. 2. illustrates the nucleotide sequence of *eryA* with corresponding translation of the three open reading frames. Standard one letter codes for the amino acids appear beneath their respective nucleic acid codons. The standard one letter codes for the amino acid sequences are as follows:

A	-alanine
R	-arginine
N	-asparagine
D	-aspartic acid
C	-cysteine
Q	-glutamine
E	-glutamic acid
G	-glycine
H	-histidine
I	-isoleucine
L	-leucine
K	-lysine
M	-methionine (start)
F	-phenylalanine

P	-proline
S	-serine
T	-threonine
W	-tryptophan
Y	-tyrosine
V	-valine

FIG. 3. is a schematic representation of Type I, Type II and Type III changes in *eryA* and structures of corresponding novel polyketides produced. Δ69 (Type I) and Δ33 (Type II) represent in-frame deletions of the base pairs in the DNA segments corresponding to the KR of module 2 and the β–ketoacyl ACP synthase of module 2, respectively. Insertion of a complete copy of module 4 within module 1 is also shown. Production of 11-epifluoro-15-norerythromycin in strain that carries Δ33 occurs when substrate analog (2S,3S,4S,5S)2,4-dimethyl-3-fluoro-5-hydroxyhexanoic acid-ethyl thioester is fed.

FIG. 4 illustrates the restriction site coordinates of cosmid pR1 5' to the sequence of *eryA* (Fig 2).

Detailed Description of the Invention

For the purposes of the present invention as disclosed and claimed herein, the following terms are defined.

The term "polyketide" as used refers to a large and diverse class of natural products, including antibiotics, pigments, and immunosuppressants. Antibiotics include, but are not limited to anthracyclines, tetracyclines, polyethers, ansamycins, macrolides of different types (polyenes and avermectins as well as classical macrolides such as erythromycins).

The term "polyketide-producing microorganism" as used herein includes any *Actinomycetales* which can produced a polyketide. Examples of Actinomycetes that produce polyketides include but are not limited to *Micromonospora rosaria*, *Micromonospora megalomicea*, *Sacharapolyspora erythraea*, *Streptomyces antibioticus*, *Streptomyces albireticuli*, *Streptomyces ambofasciens*, *Streptomyces avermitilis*, *Streptomyces fradiae*, *Streptomyces hygroscopicus*, *Streptomyces tsukubaensis*, *Streptomyces griseus*, *Streptomyces mycarofasciens*, *Streptomyces platensis*, *Streptomyces venezuelae*, *Streptomyces*

violaceoniger, and various *Actinomadura, Dactylosporangium* and
Nocardia strains that produce polyether type of polyketides.

The term "polyketide synthase" as used herein refers to the
complex of enzymatic activities responsible for the biosynthesis of
5   polyketides which include but are not limited to β-ketoreductase,
dehydratase, acyl carrier protein, enoylreductase, β-ketoacyl ACP synthase,
and acyltransferase.

The term "extender" as used herein refers to a coenzyme A
thioester of a dicarboxylate which is incorporated into a polyketide by a
10   polyketide synthase.

The term "starter" as used herein refers to a coenzyme A thioester
of a carboxylic acid which is used by the polyketide synthase as the first
building block of the polyketide.

The term "*eryA*" as used herein refers to the genes involved in the
15   formation of the polyketide moiety of erythromycin.

The term "condensation" as used herein refers to the addition of an
extender unit out to the nascent polyketide chain and requires the action
of β-ketoacyl ACP synthase, acyltransferase, and acyl carrier protein.

The term "β-carbonyl processing" as used herein refers to changes
20   effecting the carbonyl group of the growing polyketide via β-ketoreductase,
dehydratase, and enoylreductase.

The term "module" as used herein refers to the genetic element
encoding one condensation step, as defined above, and one β-carbonyl
processing step, as defined herein.

25   The term "Type I change" as used herein refers to changes in DNA
sequence which will result in the production of polyketide rings of length
identical to that of 6-deoxyerythronolide A, but with altered functional
groups at specific ring positions.

The term "Type II change" as used herein refers to alterations
30   which will result in the production of macrolide rings only when fed
exogenously with substrate analogs, e.g. thioesters of appropriate acyl
compounds of various length. Thus Type II mutants are erythromycin
non-producing (Ery⁻) mutants. The structure of the resulting macrolides
will depend on the substrate employed.

35   The term "Type III change" as used herein refers to alterations
which will result in the biosynthesis of macrolide rings of length reduced
(deletion) or increased (insertion) by two carbon units, or macrolide rings
altered in specific portions of the chain (replacement).

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In its broadest sense, the present invention entails a general procedure for producing novel polyketide structures *in vivo* by selectively altering the genetic information of the organism that naturally produces a related polyketide. A set of examples described herein are a
5   series of novel polyketides that make use of the genetic information for the biosynthesis of the polyketide portion of the macrolide antibiotic erythromycin. The organization of the segment of the *Saccharapolyspora erythraea* chromosome, designated *eryA*, and the corresponding polypeptides which it encodes that determine the biosynthesis of the
10 polyketide segment of erythromycin, are shown in FIG. 1. It is seen that *eryA* is organized in modules, as shown, and that each module takes care of one condensation step, through the action of the β-ketoacyl ACP synthase specified within, wherein an extender unit, methylmalonyl CoA, is added first to the starter unit, propionyl CoA, and then to the
15 successively growing acyl chain. The precise succession of elongation steps is dictated by the genetic order of the six modules: module 1 determines the first condensation; module 2, the second; module 3, the third, and so on until the sixth condensation step has occurred. Furthermore, the processing of the growing chain after each condensation
20 is also determined by the information within each module. Thus β-ketoreduction of the β-carbonyl takes place after each step except for step 3, as determined by the presence of a functional β-ketoreductase in all modules except module 3, whereas dehydration and enoylreduction only take place after the fourth extender unit is added to the growing acyl chain,
25 as determined by the presence of dehydratase and enoylreductase in module 4. Furthermore, the choice of the correct enantiomer (2R or 2S) of methylmalonyl-CoA as the extender unit employed at each condensation is specified by the acyltransferase function determined by each module (FIG. 1C).
30   In the present invention, novel polyketide molecules of desired structure are produced by the introduction of specific genetic alterations of the *eryA* sequence into the *Sac. erythraea* chromosome. The complete nucleotide sequence of the *eryA* segment of the *Sac. erythraea* chromosome and the sequence of the corresponding polypeptides are
35 shown in FIG. 2. Three types of alterations to the *eryA* DNA sequence are described: (i) those inactivating a single function in a module which does not arrest acyl chain growth (β-ketoreductase, dehydratase, or enoylreductase); (ii) those inactivating a single function in a module

which does arrest chain growth (β−ketoacyl ACP synthase, acyltransferase or acyl carrier protein); and (iii) those affecting an entire module (deletion, insertion, or replacement). The novel polyketides produced by strains carrying these types of mutations can be classified accordingly. Type I changes will result in the production of polyketide rings of length identical to that of 6-deoxyerythronolide A, but with altered functional groups at specific ring positions. Strains carrying type II alterations will result in the production of macrolide rings only when fed exogenously with substrate analogs, e.g.thioesters of appropriate acyl compounds of various length. Thus Type II mutants are erythromycin non-producing (Ery⁻) mutants. The structure of the resulting macrolides will depend on the substrate employed. Type III changes will result in the biosynthesis of macrolide rings of length reduced (deletion) or increased (insertion) by two carbon units, or macrolide rings altered in specific portions of the chain (replacement). A schematic representation of some examples of Type I, Type II and Type III alterations in *eryA* and the corresponding novel polyketides produced in hosts that carry such alterations is shown in FIG. 3.

In the examples described herein, specific mutations in the *eryA* region of the *Sac. erythraea* chromosome are introduced by a simple two-step approach: 1) introduction of a specified change in a cloned DNA segment; 2) exchange of the wild type allele with the mutated one. Step 1 requires standard recombinant DNA manipulations employing *E. coli* as the host. Step 2 requires one or more plasmids out of the several *E. coli*-*Sac. erythraea* shuttle vectors available and a simple screening procedure for the presence of the colony carrying the altered gene. Two methods are used to introduce the altered allele into the chromosome to replace the wild type allele. The first employs gene replacement, described in Examples 7, 11, 15, 19 and 24, wherein the gene to be altered, along with adjacent upstream and downstream DNA, is mutated and cloned into a *Sac. erythraea* non-replicating vector. The plasmid carrying the altered allele is then introduced into the host strain by transformation of protoplasts employing selection for a plasmid marker. Since the plasmid does not replicate, regenerated cells that carry the marker have undergone a single homologous recombination between one of the two segments flanking the mutation on the plasmid and its homologous counterpart in the chromosome. Some of the colonies that have subsequently lost the marker will have undergone a second recombination between the other

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plasmid borne adjacent DNA segment and its homologous chromosomal counterpart resulting in the retention of the mutation in the chromosome, replacing the normal allele with the mutant one. The second method to introduce an altered allele into the chromosome

5 employs gene conversion, described in Examples 37 and 43. In this method, an Ery⁻ *Sac. erythraea* strain carrying a deletion of a specified region of the *eryA* segment of the chromosome is used as a host. Into a *Sac. erythraea* multicopy plasmid that carries a selectable marker is cloned the wild type counterpart (segment 1) of the *eryA* segment mutant in the

10 host. Subsequently, the desired homologous or heterologous DNA segment to be introduced (segment 2) is cloned within the portion of segment 1 which is deleted in the mutant strain. The resulting plasmid is then introduced into the host employing selection for the marker. Among the transformants will be a population that have integrated

15 segments 1 and 2 from the plasmid by the process of gene conversion which can be verified by examination of the DNA among the colonies that have recovered the ability to produce erythromycin.

Two examples each of Types I, II and III alterations to the *eryA* DNA sequence and the resultant novel polyketides produced are described in

20 the examples described herein. Examples 1 through 8, 9 through 12 and 13 through 16 describe the construction and effect of three Type I mutants. Examples 17 through 22 and 23 through 27 describe the construction of two Type II mutants and the effects of feeding two different synthetic substrates to the mutant strains. Examples 28 through 38 and 39 through

25 44 outline the steps in constructing Type III changes and their respective effects on the structure of the novel polyketides produced. In Examples 1 through 7 a plasmid that contains a substantial deletion of the segment of the gene corresponding to the b-ketoreductase of module 5 is created, the altered gene is inserted into the *Sac. erythraea* chromosome to replace the

30 wild type allele and the new strain carrying the altered gene is identified and isolated. In Example 8, the new strain is fermented and the novel polyketide 5-oxo-5,6-dideoxy-3α-mycarosyl erythronolide B that results from the introduction of the mutant allele is isolated. In Examples 9 through 11, a mutation is introduced into the β–ketoreductase of module

35 2 and the mutated allele is then used to replace the wild type allele in the chromosome. In Example 12, the strain carrying the altered allele is fermented and the novel compound 11-oxo-11-deoxyerythromycin A is isolated. Similarly, in Examples 13 through 16 a mutation is introduced

into the dehydratase of module 4 and the mutated allele is then used to
replace the wild type allele in the chromosome. The strain carrying this
altered allele is then fermented and the novel products 7-
hydroxyerythromycin A and 6-deoxy-7-hydroxyerythromycin A are

5   isolated. In Examples 17 through 21, a mutation is made in the DNA
corresponding to the β-ketoacyl-ACP synthase of module 1 and introduced
into the chromosome to replace the wild type allele. This mutation has
the effect of arresting the synthesis of the polyketide chain and results in
the Ery⁻ phenotype. The synthetic substrate (2S,3R,4S,5S)3,5-dihydroxy-2,4-

10   dimethylhexanoic acid-ethyl ester is then made and fed to the mutant
resulting in the production of the novel compound (14S,15S)14(1-
hydroxyethyl)erythromycin. Similarly, in Examples 22 through 24, a
mutation is created in the β-ketoacyl-ACP synthase of module 2 and
introduced into the chromosome to replace the wild type allele. In

15   Example 25 and 26, the synthetic substrate (2S,3S,4S,5S)2,4-dimethyl-3-
fluoro-5-hydroxyhexanoic acid-ethyl thioester is made and fed to the
module 2 β-ketoacyl-ACP synthase mutant and the resulting novel
compound 11-epifluoro-15-norerythromycin is isolated. In Examples 27
through 38, a copy of the DNA sequence corresponding to module 4 is

20   introduced into the deleted segment of the β-ketoacyl-ACP synthase of
module 1 resulting in the production of the novel compound 14(1-
propyl)erythromycin. In Examples 40 through 44, a copy of the DNA
sequence corresponding to module 5 is introduced into the deleted
segment of the β-ketoacyl ACP synthase of module 1 resulting in the

25   production of the novel compound 14[1(1-hydroxypropyl)]erythromycin.

GENERAL METHODS

30   <u>Materials, Plasmids and Bacterial Strains</u>
Restriction endonucleases, T4 DNA ligase, nick-translation kit,
competent E. <u>coli</u> DH5α cells , X-gal, IPTG, and plasmids pUC19 and pUC12
are purchased from Bethesda Research Laboratories (BRL), Gaithersburg,
MD. [α-³²P]dCTP and Hybond N are from Amersham Corp., Chicago, IL.

35   Seakem LE agarose and Seaplaque low gelling temperature agarose are
from FMC Bioproducts, Rockland, ME. E. <u>coli</u> K12 strains carrying the E.
<u>coli-Sac.</u> shuttle plasmids pWHM3 or pWHM4 (Vara et al.,<u>J. Bacteriol.,</u>
171: 5872 (1989)) or the cosmids pS1 (Tuan et al.,<u>Gene</u>, 90: 21 (1990)) and

Sac. erythraea strain NRRL2338 have been deposited in the culture collection of the Agricultural Research Laboratories, Peoria, IL and are available under the accession numbers NRRL XXXX, respectively. Staphylococcus aureus Th^R (thiostrepton resistant) is obtained by plating

5   10^8 cells of S. aureus on agar medium containing 10 mg/ml thiostrepton and picking a survivor after 48 hr growth at 37°C. Thiostrepton is obtained from Squibb-Bristol Myers, New Brunswick, NJ. All other chemical and reagents are from standard commercial sources unless specified otherwise.

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DNA Manipulations

        Standard conditions (Maniatis et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982) are employed for restriction endonuclease digestion, agarose

15   gel-electrophoresis, nick translation of DNA to make ^{32}P-labeled probes, DNA ligation, and transformation of E. coli employing selection for ampicillin resistance (Ap^R) on LB agar plates. Plasmid DNA is isolated from minipreps of E. coli transformants by the boiling method (Maniatis et al., 1982, supra). DNA fragments are recovered from low melting

20   agarose gels using the method of Langridge et al., 1980. Total DNA from Sac. erythraea strains is prepared according to described procedures (Hopwood et al., Genetic Manipulation of Streptomyces, A Laboratory Manual, John Innes Foundation, Norwich, U.K., 1985). DNA is transferred from agarose gels onto Hybond N following the

25   manufacturer's instructions. Hybridizations are performed in sealed bags containing 10-20 ml of [1xNET (20xNET = 3 M NaCl, 0.3 M TrisHCl, 20 mM Na$_2$EDTA, pH 8.0), 5XDenhardt's solution (Maniatis et al., 1982, supra), 0.2 mg/ml denatured calf thymus DNA, 0.2% SDS, and 0.5-2x10^7 cpm of the nick-translated probe] for 16-20 hr at 65 ^{o}C. Filters are washed three times

30   in 1xNET/0.1% SDS for 20 min each at room temperature, and once in 0.05xNET/0.1% SDS for 20 min at 70 ^{o}C. Filters are reused as described (Donadio et al., 1990).

Amplification of DNA fragments

35   Synthetic deoxyoligonucleotides are synthesized on an ABI Model 380A synthesizer (Applied Biosystems, Foster City, CA) following the manufacturer's recommendations. Amplification of DNA fragments is performed by the polymerase chain reaction (PCR) employing a Coy

thermocycler. Reactions contain 100 pmol c   ːch primer, 1 µg of template
DNA (cosmid pS1 carrying the *eryA* segmen: ːrom Sac. erythraea strain
NRRL 2338), and 2.5 units of Thermus aquaticus DNA polymerase in a 100
ml volume of PCR buffer [50 mM KCl, 10 mM TrisHCl (pH 8.0) 2 mM

5   MgCl$_2$, 0.01% gelatin) containing 200 mM of the 4 dNTPs. The above
reagents are from Perkin Elmer Cetus, Norwalk, CT. The reaction mixture
is overlaid with a drop of paraffin oil and subjected to 30-50 cycles. Each
cycle consists of one 94 °C, one 55 °C and one 72 °C period, each of the
duration of 3 min. The progress of the amplification is monitored by

10   agarose gel-electrophoresis. The PCR primers described in the examples
below are derived from the nucleotide sequence of *eryA* of FIG. 2.

Gene replacement and gene conversion
        Protoplasts of *Sac. erythraea* strains are prepared and transformed

15   with miniprep DNA isolated from E. coli according to published
procedures (Yamamoto et al., 1986). Integrative transformants, in the case
of pWHM3 derivatives, are selected after one round of non-selective
growth of the primary Th^R transformants as described by Weber et. al,
Gene, 68: 173 (1988). Loss of the Th^R phenotype is monitored by plating

20   serial dilutions of a Th^R integrant on non-selective medium, followed by
replica-plating on thiostrepton-containing medium. Th^S (thiostrepton-
sensitive) colonies arise at a frequency of 10^{-2} (Donadio et al., 1990). The
retention of the mutant allele is established by Southern hybridization of a
few Th^S colonies.

25   A few hundred Th^R colonies obtained by transformation of an *eryA*
strain with pWHM4 derivatives are screened for antibiotic production by
the agar-plug assay employing *Staphylococcus aureus* as Th^R organism as
described (Tuan et al.,Gene, 90: 21 (1990)). The frequency of gene
conversion between a 5 kb segment of homologous sequence and a strain

30   carrying a small deletion is >25% (Tuan et al.,Gene, 90: 21 (1990)). Colonies
found to produce antibiotic activity are inoculated in SGGP (Yamamoto et
al., 1986), protoplasts are prepared, and the regenerated protoplasts are
scored for loss of the plasmid by replica-plating on non-selective medium.
Th^S colonies are then rechecked for antibiotic production, and six

35   producers are analyzed on Southern blots.

Fermentation

Sac. erythraea cells are inoculated into 100 ml SCM medium (1.5% soluble starch, 2.0% Soytone [Difco], 0.15% Yeast Extract [Difco], 0.01% CaCl₂) and allowed to grow at 32°C for 3 to 6 days. The entire culture is
5   then inoculated into 10 liters of fresh SCM medium. The fermenter is operated for a period of 7 days at 32°C maintaining constant aeration and pH at 7.0. After fermentation is complete, the cells are removed by centrifugation at 4°C and the fermentation beer is kept in the cold until further use.

10   The present invention will now be illustrated, but is not intended to be limited, by the following examples:

Example 1
Construction of plasmid pABX9

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The 9.6 kb BamHI-XhoI segment comprised between sequence coordinates 21.96 and 31.52 was isolated from cosmid pS1 and ligated to SalI-digested pUC19 DNA. The resulting mixture contained the desired plasmid pABX9.

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Example 2
Construction of E. coli K12 DH5α/pABX9

Approximately 10 ng of plasmid pABX9, prepared as described in
25   Example 1, were transformed into E. coli K12 DH5α and a few of the resulting white ApR colonies that appeared on the LB-agar plates containing X-gal and ampicillin were analyzed for their plasmid content. One colony was found to carry pABX9, as verified by the observation of fragments of 3.93, 3.39, 2.01, 1.56, 0.87, and 0.48 kb in size upon agarose gel
30   electrophoresis after SmaI digestion of the plasmid.

Example 3
Construction of plasmid pABX9DN

35   Plasmid pABX9, isolated from E. coli K12 DH5α/pABX9, was digested with NcoI and then treated with T4 DNA ligase. The resulting mixture contained the desired plasmid pABX9DN.

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Example 4
Construction of E. coli K12 DH5a/pABX9DN

Approximately 10 ng of plasmid pABX9DN, prepared as described
in Example 3, were transformed into E. coli K12 DH5α and a few of the
resulting white ApR colonies that appeared on the LB-agar plates
containing X-gal and ampicillin were analyzed for their plasmid content.
Colonies carrying pABX9DN exhibited a single NcoI fragment of 11.5 kb
visible by agarose gel electrophoresis, confirming that the 813 bp NcoI -
NcoI fragment from pABX9 has been deleted in pABX9DN.

Example 5
Construction of plasmid pABX95DN

Plasmid pABX95DN was digested with EcoRI and HindIII and
ligated to pWHM3 digested with the same two enzymes. The resulting
mixture contained the desired plasmid pABX95DN.

Example 6
Construction of E. coli K12 DH5α/pABX95DN

Approximately 10 ng of plasmid pABX95DN, prepared as described
in Example 5, were transformed into E. coli K12 DH5a and a few of the
resulting white ApR colonies that appeared on the LB-agar plates
containing X-gal and ampicillin were analyzed for their plasmid content.
Colonies carrying pABX95DN exhibited fragments of 8.8 and 7.2 kb visible
in agarose gels after EcoRI and HindIII digestion.

Example 7
Construction of Sac. erythraea AKR5 carrying the eryAKR5 allele by gene replacement

Approximately 1 mg of plasmid pABX95DN, isolated from E. coli
K12 DH5α/pABX95DN, was transformed into Sac. erythraea NRRL 2338
and stable ThR colonies were isolated. Serial dilutions of one of these
colonies were screened for the loss of the antibiotic resistance marker and
total DNA from 5 ThS colonies as well as from untransformed Sac.
erythraea NRRL 2338 was digested with SstI and analyzed by Southern

hybridization employing the 0.8 kb SalI fragment between sequence
coordinates 24.26 and 25.06 (from pABX9) as probe. Whereas NRRL 2338
showed one SstI band of 3.7 kb that hybridized to the probe, samples from
four of the Th^S strains exhibited a SstI-hybridizing band of 6.1 kb
5   indicating the presence of the mutant allele. One of these colonies was
kept and designated strain AKR5. It carries a deletion of 813 bp in the KR5
segment of *eryA* and is designated the eryAKR5 allele.

Example 8

10   Isolation, purification and properties of 5-oxo-5,6-dideoxy-3-a-mycarosyl
erythronolide B from Sac. erythraea AKR5

A 10-liter fermentation of *Sac. erythrea* AKR5 carrying the eryAKR5
allele in a Biolafitte fermentor using SNC Media. The fermentor was
15   inoculated with 100 ml of a 3 day old seed. The pO_2 was initially 80 ppm
and the temperature was maintained at 32°C. The pH was controlled to
7.0 ± 0.2 by addition of propionic acid or potassium hydroxide as needed.
At harvest (3 days), the whole broth was extracted three times with 4-liter
portions of ethylacetate. The combined extracts were concentrated under
20   reduced pressure and the residue was chromatographed on a column (50 x
5 cm) of silica gel packed and loaded in toluene and eluted with a stepwise
gradient of increasing concentration of isopropanol in toluene. Fractions
were analyzed by TLC and spots were detected by spraying with
anisaldehyde sulfuric acid spray reagent and heating. A major component
25   giving blue colored spots eluted with approximately 7% isopropanol.
Fractions containing this material were combined and concentrated to a
residue (800 mg). This was further chromatographed on a column (100 x
3 cm) of Sephadex LH-20 in chloroform-heptane-ethanol, 10:10:1, v/v/v.
Fractions were analyzed as above, early fractions (9-13) yielded 5,6-dideoxy-
30   3-a-mycarosyl-5-oxoerythronolide B (45 mg) which was crystallized from
heptane/ethylacetate mixture to mp 163-164 °C.

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CMR spectrum in CDCl3 (ppm downfield from TMS)

8.6	37.9	70.0
9.9	38.7	76.2
9.9	40.4	76.4
10.4	40.7	80.4
14.5	43.3	100.4
15.2	45.8	175.8
17.1	46.8	210.8
17.7	48.9	217.7
25.3	66.5	
25.5	69.4	

Structure was determined by single crystal X-ray diffraction.

5   Later fractions (15-17) yielded 5,6-dideoxy-5-oxoerythronolide B (10 mg) and still later fractions yielded 5,6-dideoxy-6,6a-epoxy-5-oxoerythronolide B (2.8 mg).

10   Example 9
                    Construction of plasmid pALeryAKR2

The 1.3 kb DNA segment comprised between coordinates 8.63-9.93 (fragment 1) is amplified by PCR employing two oligodeoxynucleotides, 1a
15   (5'-GGGAGCATGCTCTCGGTGCGCGGCGGCCGC-3') and 1b (5'-GCCCTGCAGCGCGTACTCCGAGGTGGCGGT-3'). Similarly, the 1.3 kb DNA segment between coordinates 9.99-11.26 (fragment 2) is PCR-amplified employing primers 2a (5'-TGGTCTGCAGGCGAGGCCGGACACCGAGG-3') and 2b (5'-
20   GGAAGAAGTCAAAGTTCCTCGGTCCCTTCT-3'). After digestion with SphI + PstI (fragment 1) and PstI + EcoRI (fragment 2), the two fragments are ligated to EcoRI + SphI-digested pWHM3. The resultant mixture contains the desired plasmid pALeryAKR2.

Example 10

Construction of E. coli K12 DH5a/pALeryAKR2

Approximately 10 ng of plasmid pALeryAKR2, prepared as
5   described in Example 9, are transformed into E. coli K12 DH5α, and a few
of the resulting white Ap[R] colonies that appear on the LB-agar plates
containing X-gal and ampicillin are analyzed for their plasmid content.
The identity of plasmid pALeryA2KR2, 9.8 kb in size, and carrying a 2.6 kb
EcoRI-SphI insert with an internal PstI site, is verified by SalI digestion
10   (fragments at 2.91, 2.21, 1.61, 1.42, 1.08, 0.29, 0.12 and 0.10 kb are released,
visible by agarose gel electrophoresis). pALeryAKR2 contains an in-frame
deletion of 102 base pairs of the corresponding segment of the wild type
eryA chromosomal DNA. The cloned segment in pALeryAKR2 is
designated the eryAKR2 allele.

15

Example 11

Construction of Sac. erythraea AKR2 carrying the eryAKR2 allele by gene
replacement

20   Approximately 1 mg of plasmid pALeryAKR2, isolated from E. coli
K12 DH5α/pALeryAKR2, is transformed into Sac. erythraea protoplasts
and stable Th[R] colonies are isolated. Serial dilutions of one of these
colonies are screened for loss of the antibiotic resistance marker, and six
Th[S] colonies are analyzed for their genotype by Southern hybridization.
25   Total DNA from the six Th[S] colonies and from untransformed Sac.
erythraea NRRL2338 is digested with PstI and with SalI and is then
examined by Southern hybridization using the 2.6 kb EcoRI-SphI insert
from pALeryAKR2 as probe. Whereas NRRL2338 contains a 39 kb PstI
hybridizing band, colonies in which the mutation in KR2 has been
30   introduced (strain AKR2) exhibit two bands of approximately equal
intensity, one at 27 kb and the other at 12 kb. The SalI digest, with bands at
1.04, 0.75, 0.29, 0.12 and 0.10 kb common to NRRL2338 and AKR2, but with
the 1.16 kb band in NRRL2338 replaced by the 1.06 kb band in AKR2,
confirms that the only change introduced into strain AKR2 is the deletion
35   of the 102 bp segment from KR2, resulting in a strain carrying the
eryAKR2 allele.

Example 12

Isolation and purification of 11-deoxy-11-oxoerythromycin A

5   The fermentation beer of strain AKR2, cooled to 4°C is adjusted to
pH 8.0 and is extracted sequentially with three equal volumes of
methylene chloride. The combined methylene extracts are concentrated to
an oily residue and partitioned between heptane and methanol. The
methanol layer is removed, washed once with heptane and concentrated
10   to a residue. The residue is digested in methylene chloride and washed
once with potassium phosphate buffer pH 7.8 and once with water. The
methylene chloride layer is concentrated to a residue and digested in the
lower phase (1:1:1, v/v/v) of a carbon tetrachloride; methanol; aqueous
phosphate buffer (0.05 M, pH 7.0) system and chromatographed on an Ito
15   Coil Planet Centrifuge in the same system. Fractions containing the
desired 11-oxo-11-deoxyerythromycin A were combined, concentrated,
digested in methylene chloride, washed well with water and concentrated
on rotary evaporator under reduced pressure to yield 11-deoxy-11-
oxoerythromycin A as an off-white solid froth. Its identity is confirmed by
comparison with antibiotic L53-18A. 11-Deoxy-11-oxoerythromycin A is
20   dissolved in tetrahydrofuran and the solution is diluted with an equal
volume of water. This is then acidified to pH 4.0 and allowed to stand at
room temperature for 4 hours. The pH is adjusted to 9.0 and the solution
is diluted with an equal volume of water and extracted with two volumes
of methylene chloride. The combined methylene chloride extracts were
25   evaporated to dryness under reduced pressure to yield antibiotic L53-18A
as a white solid.

Example 13

Construction of plasmid pALeryADH4

30
       Primers 3a (GCGCGAGCTCGACGACCAGGGCGGCATGGT) and 3b
(GGTGGCATGCTGCGACCACTGCGCGTCGGC) are used to PCR-amplify
the 1.05 kb eryA segment of the Sac. erythraea chromosome between
sequence coordinates 18.47-20.07 (fragment 3), and primers 4a
35   (AGCTGCATGCTCTGGACTGGGGACGGCTAG) and 4b
(CGCGGGATCCCAGCTCCCACGCCGATACCG) are used to amplify the
1.35 kb segment between sequence coordinates 20.58-21.96 (fragment 4) as
described in Example 1. Fragment 3 and 4, after digestion with SstI + SphI

18

and with SphI + BamHI, respectively, are ligated to SstI -, BamHI-digested
pWHM3. The resulting ligation mixture contains the desired plasmid
pALeryADH4.

5   Example 14
                  Construction of E. coli K12 DH5a/pALeryADH4

        Approximately 10 ng of pALeryADH4, prepared as described in
        Example 13, are transformed transformed into E. coli K12 DH5α, and a few
10   of the resulting white Ap^R colonies that appear on the LB-agar plates
        containing X-gal and ampicillin are analyzed for their plasmid content.
        The identity of plasmid pALeryADH4, 9.6 kb in size, is verified by SphI +
        EcoRI digestion (fragments at 7.2, 1.35 and 1.05 kb are released).
        pALeryADH4 carries a 498 base pair in-frame deletion of the
15   corresponding segment of the wild type eryA DNA. The cloned segment
        in pALeryADH4 is designated the eryADH4 allele.

                                     Example 15
        Construction of Sac. erythraea ADH4 carrying the eryADH4 allele by gene
20   replacement

        Approximately 1 mg of plasmid pALeryADH4, isolated from E. coli
        K12 DH5α/pALeryADH4, is used for transformation into Sac. erythraea
        protoplasts and stable Th^R colonies are isolated. Serial dilutions of one of
25   these colonies are screened for loss of the antibiotic resistance marker, and
        six Th^S colonies are analyzed for their genotype by Southern
        hybridization. Total DNA from the six Th^S colonies and from
        untransformed Sac. erythraea NRRL2338 is digested with SphI and with
        SstI and examined by Southern hybridization using the 2.4 kb SstI-BamHI
30   insert from pALeryADH4 as probe. Strains in which the wild type allele
        has been replaced by the mutated copy show two SphI bands, one at 13.5 kb
        and the other at 12.4 kb, whereas the wild type strain exhibits a single band
        at 26 kb. The SstI pattern, with the 2.9 kb band from NRRL2338 being
        replaced in ADH4 by a 2.5 kb band, confirms that the 487 bp deletion
35   created in plasmid pALeryADH4 has been transferred into the
        chromosome of ADH4. Strains that carry the eryADH4 allele in place of
        the wild type sequence are designated Sac. erythraea ADH4.

Example 16

Isolation and characterization of 7-hydroxyerythromycin A and 6-deoxy-7-hydroxyerythromycin A

5   The fermentation beer of strain ADH4 is cooled to 4°C and the pH is adjusted to 5.0. The mixture is extracted once with an equal volume of methylene chloride. The pH of the aqueous layer is readjusted to 9.0 and two further methylene chloride extracts are carried out. These two extracts are combined, washed with water and concentrated to a residue. This is

10   digested in 10 ml of the upper phase of a (3:7:5, v/v/v) mixture of hexane, ethylacetate, aqueous phosphate buffer (0.05 M, pH 7.5) and chromatographed on an Ito Coil Planet Centrifuge in the same system. Fractions containing the desired 7-hydroxyerythromycin were combined, concentrated, and partitioned between methylene chloride and dilute (pH

15   9.5) ammonium hydroxide solution. Fractions containing the desired 6-deoxy-7-hydroxyerythromycin were combined, concentrated, and partitioned between methylene chloride and dilute (pH 9.5) ammonium hydroxide solution. The methylene chloride layers are washed with water and then concentrated to yield the desired 7-hydroxyerythromycin A and

20   6-deoxy-7-hydroxyerythromycin A as white foams.

Example 17

Construction of plasmid pALeryAKS1

25   The 1.4 kb segment of *eryA* , between sequence coordinates 1.11-2.54 (fragment 5) and the 1.5 kb segment between sequence coordinates 2.88-4.37 (fragment 6) are PCR-amplified using primers 5a (TGCAGAATTCGCTGGCCGCGCTCTGGCGCT) and 5b (GAGAGCTGCAGCATGAGCCGCTGCTGCGGG), and 6a

30   (CATGCTGCAGGACTTCAGCCGGATGAACTC) and 6b (GAGGAAGCTTCCAGCCGGTCCAGTTCGTCC), respectively, as described in Example 9. After digestion with EcoRI + PstI (fragment 5) and PstI + HindIII (fragment 6), the two fragments are ligated to EcoRI + HindIII-digested pWHM3. The resulting mixture contains the desired plasmid

35   pALeryAKS1.

20

Example 18
Construction of E. coli K12 DH5a/pALeryAKS1

Approximately 10 ng of pALeryAKS1, prepared as described in
5 Example 17, are transformed into E. coli K12 DH5α, and a few of the
resulting white Ap^R colonies that appear on the LB-agar plates containing
X-gal and ampicillin are analyzed for their plasmid content. The identity
of plasmid pALeryAKS1, 10.1 kb in size, is verified by digestion with PstI
+ HindIII (fragments of 8.6 and 1.5 kb are observed by agarose gel
10 electrophoresis) and with SalI (fragments of 2.93, 2.21, 1.42, 1.37, 0.86, 0.54,
0.27, 0.14, 0.13, and 0.10 kb are observed). pALeryAKS1 carries an in-frame
deletion of 282 base pairs of the corresponding wild type eryA DNA. The
cloned insert in plasmid pALeryAKS1 is designated the eryAKS1 allele.

15 ## Example 19
Construction of Sac. erythraea AKS1 carrying the eryAKS1 allele by gene replacement

Approximately 1 mg of plasmid pALeryAKS1, isolated from E. coli
20 K12 DH5α/pALeryAKS1, is used for transformation into Sac. erythraea
protoplasts and stable Th^R colonies are isolated. Serial dilutions of one of
these colonies are screened for loss of the antibiotic resistance marker, and
six Th^S colonies are analyzed for their genotype by Southern
hybridization. Total DNA from the six Th^S colonies and from
25 untransformed Sac. erythraea NRRL2338 is digested with PstI and with
SmaI and examined in Southern hybridization employing the 2.9 kb
EcoRI-HindII insert from pALeryAKS1 as probe. Colonies in which the
wild type allele has been replaced by the mutated copy (strain AKS1) show
two PstI bands, one at 34.5 and the other at 4.4 kb, whereas the wild type
30 strain exhibits a single band at 39 kb. The SmaI pattern, with the 2.9 kb
band from NRRL2338 being replaced in AKS1 by a 2.6 kb band, confirms
that the 282 bp created in plasmid pALeryAKS1 has been transferred into
strain AKS1. Strains that carry the eryAKS1 allele are designated Sac.
erythraea AKS1.
35

21

Example 20

Synthesis of (2S,3R,4S,5S)3,5-dihydroxy-2,4-dimethylhexanoic acid n-butyl thioester

5   A convenient source of this compound in chiral purity is the antibiotic oleandomycin. Oleandomycin (5 g) is dissolved in an aprotic solvent such as toluene and treated with diazabicyclo[5.4.0]undecene-5 (1 g) and heated for one hour. The resulting solution is poured into iced water, agitated well and the organic layer is drawn off and concentrated to

10   a residue. The residue is digested in methylene chloride and treated exhaustively with a solution of ozone. The resulting ozonide is oxidatively decomposed with dilute hydrogen peroxide in sufficient aqueous ethanol to yield a monophasic mixture. This is further diluted with water and made 0.1 N with sodium hydroxide. The mixture is

15   warmed for one hour at 70°C and then cooled before being acidified to pH 2.5 with dilute sulfuric acid. The mixture is then exhaustively extracted with methylene chloride. The combined extracts are concentrated to an oily residue and the desired lactone is recovered by chromatography on silica gel eluted with a gradient of toluene-

20   isopropanol.
         The δ-lactone is converted to the butyl thioester before feeding to Sac. erythrea AKS1 by refluxing with n-butylthiol in the presence of a catalytic amount of triethylamine.

25   Example 21

Isolation of (14S,15S)14(1-hydroxyethyl)erythromycin A

         The fermentation broth of AKS1 is cooled to 4°C and adjusted to pH 4.0 and extracted once with methylene chloride. The aqueous layer is

30   readjusted to pH 9.0 and extracted twice with methylene chloride and the combined extracts are concentrated to a solid residue. This is digested in methanol and chromatographed over a column of Sephadex LH-20 in methanol. Fractions are tested for bioactivity against a sensitive organism, such as Staphylococcus aureus Th^R, and active fractions are combined.

35   The combined fractions are concentrated and the residue is digested in 10 ml of the upper phase of a solvent system consisting of n-heptane, benzene, acetone, isopropanol, 0.05 M, pH 7.0 aqueous phosphate buffer (5:10:3:2:5, v/v/v/v/v), and chromatographed on an Ito Coil Planet

Centrifuge in the same system. Active fractions are combined,
concentrated and partitioned between methylene chloride and dilute
ammonium hydroxide (pH 9.0). The methylene chloride layer is
separated and concentrated to yield the desired product as a white foam.

5

Example 22
Construction of plasmid pALeryAKS2

Primers 7a (CGCCCGAATTCGAGGCGCTGGGCGCCCGGAC) and 7b
10 (CCACCTGCAGCGCGGGACCTTCCAGCCCC), and primers 8a
(GTGGGTCGCTGCAGACGGTGACTGCGG) and 8b
(GGTCAAGCTTCGTCGGCGAGCAGCTTCTC) are used to PCR-amplify
the 1.45 kb *eryA* segment between sequence coordinates 5.71-7.16
(fragment 7) and the 1.5 kb *eryA* segment between sequence coordinates
15 7.22-8.70 (fragment 8), respectively. After digestion with EcoRI + PstI
(fragment 7) and with PstI + HindIII (fragment 8), the two fragments are
ligated to pWHM3 cut with EcoRI + HindIII. The resulting mixture
contains the desired plasmid pALeryAKS2.

20

Example 23
Construction of E. coli K12 DH5a/pALeryAKS2

Approximately 10 ng of pALeryAKS2, prepared as described in
Example 22, are transformed into E. coli K12 DH5α, and a few of the
25 resulting white Ap^R colonies that appear on the LB-agar plates containing
X-gal and ampicillin are analyzed for their plasmid content. The identity
of plasmid pALeryAKS2, 10.1 kb in size, is verified by digestion with PstI
+ HindIII (fragments of 8.6 and 1.5 kb are observed by agarose gel
electrophoresis) and with SstII (fragments of 4.0, 2.3, 2.0, 0.72, 0.43, 0.40,
30 0.20, 0.18, 0.13 and 0.11 kb observed). Plasmid pALeryAKS2 carries an in-
frame deletion of 60 base pairs of the corresponding wild type *eryA* DNA.
This deletion removes the active site cysteine from KS2. The cloned
insert in plasmid pALeryAKS2 is designated the *eryAKS2* allele.

Example 24

Construction of Sac. erythraea AKS2 carrying the eryAKS2 allele by gene replacement

5　　　Approximately 1 mg of plasmid pALeryAKS2, isolated from E. coli
K12 DH5α/pALeryAKS2, is used for transformation into Sac. erythraea
protoplasts and stable Th^R colonies are isolated. Serial dilutions of one of
these colonies are screened for loss of the antibiotic resistance marker, and
six Th^S colonies are analyzed for their genotype by Southern
10　hybridization. Total DNA from the six Th^S colonies and from
untransformed Sac. erythraea NRRL2338 is digested with PstI and with
SstII and examined in Southern hybridization employing the 2.9 kb EcoRI-
HindII insert from pALeryAKS2 as probe. Colonies in which the wild type
allele has been replaced by the mutated copy (strain AKS2) show two PstI
15　bands, one at 34.5 and the other at 4.4 kb, whereas the wild type strain
exhibits a single band at 39 kb. The SstII pattern, with the 0.78 kb band
from NRRL2338 being replaced in AKS2 by a 0.72 kb band, confirms that
the 60 bp created in plasmid pALeryAKS2 has been transferred into strain
AKS2. Strains that carry the eryAKS2 allele are designated Sac. erythraea
20　AKS2.

Example 25

Synthesis of (2R,3R,4S,5R)2,4-dimethyl-3-fluoro-5-hydroxyhexanoic acid n-butyl thioester

25

　　　(2R,3S,4S,5R)3,5-Dihydroxy-2,4-dimethylhexanoic acid-δ-lactone (1
g) from Example 20 is digested in 10 ml of pyridine and treated with p-
toluenesulfonyl chloride (1.3 g) and allowed to stand at room temperature
overnight. The mixture is poured into iced water and extracted with
30　methylene chloride and the methylene chloride is concentrated to the
crude sulfonate ester. This is digested in acetonitrile (100 ml) and heated
under reflux after the addition of tetrabutylammonium fluoride (1.75 g).
After 6 hours the mixture is cooled, poured over iced water (300 ml) and
extracted three times with 200 ml portions of methylene chloride. The
35　combined methylene chloride extracts were concentrated and the residue
was chromatographed on a column of silica gel eluted with a stepwise
gradient of isopropanol (0 to 50%) in toluene. Fractions containing
(2R,3R,4S,5R)2,4-dimethyl-3-fluoro-5-hydroxyhexanoic acid δ-lactone were

combined and concentrated to a white solid. The lactone is then converted to the n-butyl thiolester by refluxing in n-butyl thiol with a catalytic amount of triethylamine. Solvent is removed and the residue is digested in DMSO before feeding to fermentations of Sac. erythraea AKS2.

Example 26
Isolation and purification of 11-epifluoro-15-norerythromycin A

The fermentation broth of strain AKS2 is cooled to 4°C and adjusted to pH 4.0 and extracted once with ethylacetate. The aqueous layer is adjusted to pH 9.0 and extracted twice with methylene chloride and the combined extracts are concentrated to a white solid. This is chromatographed over a column of Sephadex LH-20 in a mixture of heptane, chloroform, ethanol (10:10:1, v/v/v) and fractions containing the desired product are combined and concentrated to a solid residue. This is further purified by countercurrent chromatography on an Ito Coil Planet Centrifuge on a system composed of carbon tetrachloride; methanol; 0.05 M; pH 7.0 aqueous potassium phosphate buffer (1:1:1, v/v/v). Fractions containing the desired 11-epifluoro-15-norerythromycin were combined, and concentrated to a residue. This was digested in methylene chloride and dilute (pH 9.5) ammonium hydroxide and the methylene chloride layer was separated, washed with water and concentrated to yield the desired 11-epifluoro-15-norerythromycin A as white solid.

Example 27
Construction of plasmid pALeryAM4.1

Primers 9a (GCGCCGAATTCTCGAGACGGCGTGGGAGGCA) and 9b (TTGCGGTACCAGTAGGAGGCGTCCATCGCG) are employed to PCR-amplify the 2.0 kb eryA segment between sequence coordinates 17.35-19.38 (fragment 9). After digestion with EcoRI + KpnI, fragment 9 is ligated to pUC19 cut with the same two enzymes. The resulting mixture contains the desired plasmid pALeryAM4.1.

25

Example 28
Construction of E. coli K12 DH5a/pALeryAM4.1

5   Approximately 10 ng of pALeryAM4.1, prepared as described in
Example 27, are transformed into E. coli K12 DH5a, and a few of the
resulting white Ap[R] colonies that appear on the LB-agar plates containing
X-gal and ampicillin are analyzed for their plasmid content. The identity
of plasmid pALeryAM4.1, 4.7 kb in size, is verified by digestion with SalI
(fragments of 2.8, 0.85, 0.53, 0.27 and 0.22 kb are observed by agarose gel
10   electrophoresis).

Example 29
Construction of plasmid pALeryAM4.2

15   Primers 10a (GCTGGGATCCCGCGGCGCGGGTTGCAGCAC) and
10b (CGGAACTCGGTGAGCATGCCGGGACTGCTC) are used to PCR-
amplify the 2.1 kb eryA segment between sequence coordinates 21.94-24.00
(fragment 10). The 2.6 kb fragment KpnI(96)-BamHI(102) from cosmid
clone pR1, and fragment 10 cut with BamHI + SphI, are ligated to
20   pALeryAM4.1 cut with KpnI + SphI. The resulting mixture contains the
desired plasmid pALeryAM4.2.

Example 30
Construction of E. coli K12 DH5a/pALeryAM4.2

25

        Approximately 10 ng of pALeryAM4.2, prepared as described in
Example 29, are transformed into E. coli K12 DH5a, and a few of the
resulting white Ap[R] colonies that appear on the LB-agar plates containing
X-gal and ampicillin are analyzed for their plasmid content. The identity
30   of plasmid pALeryAM4.2, 9.3 kb in size, is verified by digestion with XhoI
+ SphI (to ensure that the entire 6.65 kb insert is released) and with SalI,
with fragments of 2.8, 1.82, 1.09, 0.94, 0.85, 0.75, 0.45, 0.27, 0.22 and 0.13 kb
are observed by agarose gel electrophoresis).

Example 31
Construction of plasmid pALeryAM1

The 2.9 kb SmaI(4)-SmaI(20) fragment from cosmid clone pR1 is
ligated to pUC12 cut with SmaI. The resulting mixture contains plasmid
pALeryAM1.

Example 32
Construction of E. coli K12 DH5αa/pALeryAM1

Approximately 10 ng of pALeryAM1, prepared as described in
Example 31, are transformed into E. coli K12 DH5α, and a few of the
resulting white ApR colonies that appear on the LB-agar plates containing
X-gal and ampicillin are analyzed for their plasmid content. The identity
of plasmid pALeryAM1, 5.6 kb in size, is verified by digestion with SmaI
(the 2.9 kb insert is realeased) and with SphI, with release of one 4.4 and
one 1.07 kb bands. Both orientations of the insert in plasmid pALeryAM1
are useful.

Example 33
Construction of plasmid pALeryAM4.3

Plasmid pALeryAM1 is cut with XhoI to completion, partially with
SphI, and the resulting 5.25 kb band, isolated from an agarose gel, is ligated
to the 6.65 kb insert released from pALeryAM4.2 by XhoI + SphI digestion
The resulting mixture contains the desired plasmid pALeryAM4.3.

Example 34
Construction of E. coli K12 DH5a/pALeryAM4.3

Approximately 10 ng of pALeryAM4.3, prepared as described in
Example 33, are transformed into E. coli K12 DH5α, and a few of the
resulting white ApR colonies that appear on the LB-agar plates containing
X-gal and ampicillin are analyzed for their plasmid content. The identity
of plasmid pALeryAM4.1, 11.9 kb in size, is verified by XhoI + SphI
digestion (fragments of 6.65 and 5.25 kb are visible by agarose gel-
electrophoresis). Plasmid pALeryAM4.3 carries the entire eryA module 4

27

inserted into the KS region of module 1. The cloned insert in
pALeryAM4.3 is degnated the eryAM412 allele.

Example 35
Construction of plasmid pALeryAM4.4

Plasmid pALeryAM4.3 is cut with EcoRI + HindIII, and the
resulting 9.2 kb band, recovered from an agarose gel, is ligated to pWHM4
cut with the same two enzymes. The resulting mixture contains the
desired plasmid pALeryAM4.4.

Example 36
Construction of E. coli K12 DH5α/pALeryAM4.4

Approximately 10 ng of pALeryAM4.4, prepared as described in
Example 35, are transformed into E. coli K12 DH5α, and a few of the
resulting white Ap^R colonies that appear on the LB-agar plates containing
X-gal and ampicillin are analyzed for their plasmid content. The identity
of plasmid pALeryAM4.1, 16.5 kb in size, is verified by EcoRI + HindIII
digestion, with fragments of 9.2 and 7.3 kb released. Plasmid
pALeryAM4.4 carries the eryAM412 allele on the Sac. erythraea multicopy
vector pWHM4.

Example 37
Construction of Sac. erythraea AM412 carrying the eryAM412 allele by
gene conversion

Approximately 1 mg of plasmid pALeryAM4.4, isolated from E. coli
K12 DH5α/pALeryAM4.4, is used for transformation into Sac. erythraea
strain AKS1 protoplasts. A few hundred transformants are screened for
antibiotic production by the agar-plug assay, and one of the colonies found
to produce antimicrobial activity is cured of pALeryAM4.4 by protoplast
formation and regeneration as described in General Methods. Total DNA
from six antibiotic-producing, Th^S colonies (strain AM412) and from strain
AKS1 is digested with SphI and with XhoI and the resulting Southern blot
is hybridized first to the 2.9 kb insert from pALeryAM1, and then to the 2.9
kb SstI(95)-SstI(101) fragment from plasmid pALeryAM4.2. With the first
probe, the SphI band at 0.8 kb in strain AKS1 is seen to be replaced by a 7.5

kb band in strain AM412, whereas the other two bands at 2.4 kb and 5.2 kb
are unaffected. In the XhoI digest, the AKS1 band at 2.9 kb is replaced by a
9.6 kb band in AM412, with the other band at 5.2 kb conserved in both
strains. Using the SstI(95)-SstI(101) fragment as probe, strain AKS1
exhibits one band at 25.5 kb and one at 17.9 kb in the SphI and XhoI digests,
respectively, whereas, in addition to these bands, strain AM412 shows one
SphI band at 7.5 kb and one XhoI band at 9.6 kb. In this way, it is
established that the eryAKS1 allele has been converted into the eryAM412
allele in strain AM412.

Example 38
Isolation and purification of 14-(1-propyl)erythromycin A

At harvest the fermentation is adjusted to pH 9.5 and extracted
twice with equal volumes of methylene chloride. The combined extracts
are washed once with water and concentrated to an oily residue. This is
partitioned in a heptane methanol water (5:5:1, v/v/v) system and the
lower layer is washed once with heptane and then concentrated to a
semisolid residue. This is digested in methanol and chromatographed
over a column of Sephadex LH-20 in methanol. Fractions are tested for
bioactivity in an agar diffusion assay on plates seeded with the macrolide-
sensitive strain Staphylococcus aureus Th^R. Active fractions are
combined and further purified by chromatography over silica gel a
chloroform:methanol gradient containing 0.1% triethylamine. Fractions
containing the desired 14-(1-propyl)erythromycin A are combined and
concentrated to yield the product as a white solid.

Example 39
Construction of plasmid pALeryAM5.1

The 4.7 kb eryA fragment between sequence coordinates 23.65-28.36
(fragment 11) is PCR-amplified employing primers 11a
(ATGCTCGAGATCTCGTGGGAGGCGCTGGA) and 11b
(AGAACTCGGTGAGCATGCCCGGGCCCGCCA). Fragment 11, after
digestion with XhoI + SphI, is ligated to the 5.25 kb fragment resulting
from complete XhoI and partial SphI digestion of pALeryAM1, as in
Example 33. The resulting mixture contains the desired plasmid
pALeryAM5.1.

Example 40
Construction of E. coli K12 DH5α/pALeryAM5.1

5   Approximately 10 ng of pALeryAM5.1, prepared as described in
Example 39, are transformed into E. coli K12 DH5α, and a few of the
resulting white Ap^R colonies that appear on the LB-agar plates containing
X-gal and ampicillin are analyzed for their plasmid content. The identity
of plasmid pALeryAM5.1, 9.95 kb in size, is verified by SphI + XhoI
10   digestion, with fragments of 5.25 and 4.7 kb released, and by SmaI
digestion where fragments of 3.39, 2.68 and 1.94 (doublet) kb are observed.
Plasmid pALeryAM5.1 carries the entire eryA module 5 inserted into the
β–ketoacyl ACP synthase region of module1. The cloned insert in plasmid
pALeryAM5.1 is designated the eryA512 allele.

15

Example 41
Construction of plasmid pALeryAM5.2

20   Plasmid pALeryAM5.1 is cut with EcoRI + HindIII and the resulting
6.3 kb fragment, recovered from an agarose gel, is ligated to pWHM4 cut
with the same two enzymes. The resulting mixture contains the desired
plasmid pALeryAM5.2.

Example 42
25   ### Construction of E. coli K12 DH5α/pALeryAM5.2

        Approximately 10 ng of pALeryAM5.2, prepared as described in
Example 41, are transformed into E. coli K12 DH5α, and a few of the
resulting white Ap^R colonies that appear on the LB-agar plates containing
30   X-gal and ampicillin are analyzed for their plasmid content. The identity
of plasmid pALeryAM5.2, 13.6 kb in size, is verified by digestion with
EcoRI + HindIII, with fragments of 7.3 and 6.3 kb released. Plasmid
pALeryAM5.2 contains the eryAM512 allele on the Sac. erythraea
multicopy vector pWHM4.

35

Example 43
Construction of Sac. erythraea AM512 carrying the eryAM512 allele by
gene conversion

5   Approximately 1 mg of plasmid pALeryAM5.2, isolated from E. coli
K12 DH5α/pALeryAM5.2, is used for transformation into Sac. erythraea
strain AKS1 protoplasts. A few hundred transformants are screened for
antibiotic production by the agar-plug assay, and one of the colonies found
to produce antimicrobial activity is cured of pALeryAM5.2 by protoplast
10   formation and regeneration as described in General Methods. Total DNA
from six antibiotic-producing, Th^S colonies (strain AM512)and from strain
AKS1 is digested with SphI and with XhoI and the resulting Southern blot
is hybridized first to the 2.9 kb insert from pALeryAM1, and then to the 0.8
kb NcoI(119)-NcoI(123) fragment from plasmid pALeryAM5.1. With the
15   first probe, the SphI band at 0.8 kb in strain AKS1 is replaced by a 5.5 kb
band in strain AM512, whereas the other two bands at 2.4 kb and 5.2 kb are
unaffected. In the XhoI digest, the AKS1 band at 2.9 kb is replaced by a 7.6
kb band in AM512, with the other band at 5.2 kb conserved in both strains.
Using the NcoI(119)-NcoI(123) fragment as probe, strain AKS1 exhibits one
20   band at 25.5 kb and one at 17.9 kb in the SphI and XhoI digests,
respectively, whereas,.in addition to these bands, strain AM512 shows one
SphI band at 5.5 kb and one XhoI band at 7.6 kb. In this way, it is
established that the eryAKS1 allele has been converted into the eryAM512
allele in strain AM512.

25
Example 44
Isolation and purification of 14[1(1-hydroxypropyl)]erythromycin A

At harvest the pH of the fermentation of AM512 is adjusted to 9.5
30   and the mixture is extracted twice with equal volumes of ethylacetate.
The combined ethylacetate extracts are washed with water, dried and
partitioned in a heptane, methanol, water (5:5:1, v/v/v) system. The
lower (methanolic phase) is washed with an equal volume of heptane and
is concentrated to a residue. This is chromatographed on a Sephadex LH-
35   20 column in methanol and fractions containing the desired 14[1(1-
hydroxypropyl)]erythromycin A are concentrated and further purified by
chromatography on an Ito Coil Planet Centrifuge in a system consisting of
n-heptane, benzene, acetone, isopropanol, 0.65 M, pH 7.0 aqueous

potassium phosphate buffer (5:10:2:3:5, v/v/v/v/v). Fractions containing
the desired product are concentrated to a solid residue and partitioned
between methylene chloride and dilute (pH 9.5) ammonium hydroxide.
The organic layer is washed with water and concentrated to yield 14[1(1-
5   hydroxypropyl)]erythromycin A as a white solid.

        Although the present invention is described in the Examples listed
above in terms of preferred embodiments, they are not to be regarded as
limiting the scope of the invention. The above descriptions serve to
10 illustrate the principles and methodologies involved in creating the three
types of mutations that can be introduced into the *eryA* segment of the
Sac. erythraea chromosome that result in the synthesis of novel
polyketide products. Although single Type I alterations, leading to the
production of 5-oxo-5,6,-dideoxy-3α-mycorosyl erythronolide B, 11-oxo-11-
15 deoxyerythromycin A, 7-hydroxyerythromycin A, 7-oxo-
7deoxyerythromycin A, 5-desosaminyl-3-oxo-3-deoxyerythronolide A, and
Δ-6,7-anhydro-6-deoxyerythromycin A are specified herein, it is obvious
that other Type I changes can be introduced into the *eryA* segment
leading to novel polyketide structures. Among the additional Type I
20 alterations that can be obtained are those in which two or more modules
are affected leading to the synthesis of novel polyketides. Examples of
combinations of two Type I alterations leading to useful compounds
include but are not limited to: mutants of the the β-ketoreductase of
module 2 (KR2) and the β-ketoreductase of module 4 (KR4) leading to the
25 formation of 7,11-dioxo-7,11-dideoxyerythromycin A; mutants of KR2 and
the β-ketoreductase of module 6 (KR6) leading to the formation of 3,11-
dioxo-3,11-dideoxy-5-desosaminylerythronolide A; mutants of KR2 and
the dehydratase of module 4 (DH4) leading to the synthesis of 7-hydroxy-
11-oxo-11-deoxyerythromycin A; mutants of KR2 and the enoylreductase
30 of module 4 (ER4) leading to the synthesis of Δ-6,7-anhydro-11-oxo-11-
deoxyerythromycin A; mutants of KR4 and KR6 leading to the synthesis of
3,7-dioxo-3,7-dideoxy-5-desosaminylerythronolide A; mutants of KR6 and
DH4 leading to the synthesis of 3-oxo-3-deoxy-5-desosaminyl-7-
hydroxyerythronolide A; mutants of KR6 and ER4 leading to the synthesis
35 of 3-oxo-3-deoxy-5-desosaminyl-Δ-6,7-anhydroerythronolide A. Examples
of combinations of three Type I alterations leading to the synthesis of
novel polyketides include but are not limited to: mutants of KR2, KR4 and
KR6 leading to the synthesis of 3,7,11-trioxo-3,7,11-trideoxy-5-

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desosaminylerythronolide A; mutants of KR2, KR6 and DH4 leading to
the synthesis of 3,11-dioxo-3,11-dideoxy-5-desosaminyl-7-
hydroxyerythronolide A; mutants of KR2, KR6 and ER4 leading to the
synthesis of 3,11-dioxo-3,11-dideoxy-5-desosaminyl-D-6,7-

5   anhydroerythronolide A. All combinations of two or three Type I
mutants, the Sac. erythraea strains that carry said combinations and the
corresponding polyketides produced from said strains, therefore, are
included within the scope of the present invention.

      Although the Type II mutants specified herein have been

10 constructed in the β–ketoacyl ACP synthase of module 1 (KS1) and the β–
ketoacyl ACP synthase of module 2 (KS2), other Type II mutants can be
constructed in other domains to result in the synthesis of novel
polyketide structures upon feeding with appropriate substrate analogs.
Other Type II mutants include but are not limited to: inactivation of the

15 either of the acyltransferases or acyl carrier proteins of module 1, or the
acyltransferase or acyl carrier protein of module 2, the β–ketoacyl ACP
synthase, acyltransferase or acyl carrier protein of module 3, module 4 or
module 5. Furthermore, compounds other than (2S,3R,4S,5S)3,5-
dihydroxy-2,4-dimethylhexanoic acid-ethyl thioester and (2S,3S,4S,5S)2,4-

20 dimethyl-3-fluoro-5-hydroxyhexanoic acid-ethyl thioester specified herein
can be synthesized and fed to strains AKS1 or AKS2 specified herein or
other strains that carry other Type II mutations to result in the creation of
novel polyketides that are within the scope of the present invention.

      Although two examples of Type III alterations are specified herein,

25 it is apparent to those skilled in the art that many other examples of Type
III changes are possible. Strains of Sac. erythraea carrying changes of this
type offer the very high potential for the production of novel polyketides
of specified structure, since they do not require synthetic substrates as do
Type II mutants and they are not limited to the formation of derivatives

30 of erythromycin, as in the case of Type I mutants. In the embodiments of
Type III mutants specified herein, we have illustrated how a second copy
of a complete module can be introduced at a desired position by gene
conversion to result in the synthesis of 14-(1-propyl)erythromycin A or 14-
[1(1-hydroxypropyl))erythromycin A. These alterations make use of the

35 high conservation and simultaneous lack of specificity of the β–ketoacyl
ACP synthases of modules 1 and 2, thereby making possible the
construction of hybrid β–ketoacyl ACP synthase functions consisting of
portions of proteins derived from different modules. Those skilled in the

art understand, therefore, that it is possible, as exemplified for KS1 and
KS2, to delete a small portion of the β–ketoacyl ACP synthase of other
modules and to construct strains carrying such alterations which can then
be employed as hosts for introducing at the deleted β–ketoacyl ACP
synthase location a second copy of any homologous module.
Furthermore, as exemplified herein, it is also possible to delete any
segment of *eryA* by ligation of two non-contiguous PCR-generated
fragments and to subsequently construct strains, therefore, devoid of any
or all portions of any module. Such strains deleted of a full module can be
employed for reintroduction of either the same or a different module at a
different location. It is possible, therefore, to determine the novel
structures desired and then create a series of *Sac. erythraea* strains
containing the corresponding arrangements of *eryA* modules that would
produce said novel structures that are included within the scope of the
present invention. Additional examples of novel compounds produced
from the construction of Type III alterations include but are not limited to
11-deoxyerythromycin, resulting from the insertion of the *eryA* segment
encoding DH4 and ER4 in module 2.

Moreover, it will also be apparent that two or more modules can be
excised and introduced into various sites of the *Sac. erythraea*
chromosome to produce novel polyketides of predicted structure such as
the introduction of the *eryA* segment encoding DH4 and ER4 in both
module 1 and module 2 to result in the production of 14(R)[1-
hydroxypropyl]11-deoxyerythromycin A. All combinations, therefore, of
Type III alterations and the strains of *Sac. erythraea* that carry said
alterations as well as the polyketides produced from said strains are
included within the scope of the present invention.

In addition, it is also possible to create combinations of Type I, Type
II and Type III alterations and insert such alterations into *Sac. erythraea* to
produce novel polyketides. Examples of such combinations include but
are not limited to the following. The combination of a Type I alteration,
such as an alteration in DH4 and a Type II alteration, such as a mutation
in the KS1 to result in the formation of (14S,15S)14-[1-hydroxyethyl]-7-
hydroxyerythromycin A when the strain of *Sac. erythraea* carrying such
alterations is fed with the compound (2S,3R,4S,5S)3,5-dihydroxy-2,4-
dimethylhexanoic acid ethyl ester. The combination of a Type I alteration,
such as an alteration in DH4 and a Type III alteration, such as found in
Sac. erythraea strain AM412, wherein a copy of the DNA segment of

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module 4 is introduced in module 1, such that the *Sac. erythraea* strain so constructed produces the compound 7-hydroxy-14-propylerythromycin A. All combinations of two or more alterations of Type I, Type II and Type III alterations, the *Sac. erythraea* strains that carry such alterations, and the

5   polyketides produced from such strains are included within the scope of the present invention. It will also occur to those skilled in the art that novel structures can be produced by altering the specificity of the acyltransferase functions in any module. Examples include: replacement of the acyltransferase domains of modules 1, 2, 3, 4, 5,or 6 in *eryA* with

10   those of modules 4, 4, 2, 2, 2, and 4, respectively, to result in the production of 12-epierythromycin A,10-epierythromycin A, 8-epierythromycin A, 6-epierythromycin A, 4-epierythromycin A and 2-epierythromycin A, respectively, that are included within the scope of the present invention.

15   It should be emphasized that the introduction of an entire *eryA* module at a different location, as exemplified for the construction of *Sac. erythraea* strains AM412 and AM512 in Examples 29 and 35, respectively, does not rely on homologous recombination between the incoming *eryA* module and the host chromosome. Rather, gene conversion of the host

20   allele with the *eryA* allele residing on the multicopy plasmid requires DNA sequences homologous to the host allele flanking the incoming module. Thus, any module carrying the desired specificities, either from homologous or heterologous sources, can be employed in gene conversion of the host allele, provided that is flanked by segments of

25   homology. It will occur to those skilled in the art, therefore, that, given the large number of natural polyketide molecules existing, a wide variety of additional novel molecules of predicted structure can be produced in Type III mutants containing an additional module of desired specificities or where an endogenous module is replaced by an exogenous one. The

30   length of the acyl chain can be easily controlled by suitably changing the number of modules involved in its synthesis. Similarly, the introduction of keto, hydroxy, enoyl, or methylene groups at specific points along the acyl chain can be easily achieved by introducing the proper b-carbon processing functions (β-ketoreductase, dehydratase and enoylreductase) in

35   the required modules. Exogenous modules constitute the source of specificities for starter and extender units other than those employed by *Sac. erythraea* for erythromycin biosynthesis, making it thereby possible to employ, for example, malonylCoA or (2R)- or (2S)ethylmalonylCoA, etc.

as extender units, and acetyl CoA, butyryl CoA, etc. as the starter unit. The
result will be the formation of erythromycin analogs containing the
desired functional groups and side chains with the desired
stereochemistry. As an extension of the examples reported with *eryA*, the

5   construction of a *Sac. erythraea* strain carrying a heterologous module
inserted into *eryA* requires: (i) cloning of the genes from any other
<u>Actinomyces</u> producing a polyketide with desired structural features; (ii)
mapping of the modular organization of the cloned genes by low
stringency hybridization and restriction analysis; (iii) locating the module

1 0 carrying the desired specificities by partial sequencing; (iv) precise excision
of the desired genetic element and cloning into a vector suitable for gene
conversion; (v) construction and transformation of a *Sac. erythraea* strain
suitable for gene conversion and screening for the novel compound. Any
module, or portion thereof, can thus be precisely excised from the genome

1 5 of a polyketide-producing microorganism and introduced into suitable
Sac. erythraea strains to create a novel polyketide of predicted structure.
Thus, replacement of the acyltransferase segments of modules 1, 2, 3, 4,
5,or 6 in *eryA* with the acyltransferase segment specific for malonyl CoA,
such as can be found in the polyketide synthase genes for the synthesis of

2 0 pikromycin in <u>Streptomyces venezuelae</u>, to result in the synthesis of 12-
norerythromycin A, 10-norerythromycin A, 8-norerythromycin A, 6-
norerythromycin A, 4-norerythromycin A and 2-norerythromycin A,
respectively, that are included within the scope of the present invention.
In addition, replacement of the acyltransferase segments of modules 1, 2, 3,

2 5 4, 5,or 6 in *eryA* with an acyltransferase specific for (2R)-ethylmalonyl
CoA, such as can be found in the polyketide synthase genes for the
synthesis of spiramycin in <u>Streptomyces ambofasciens</u>, will result in the
formation of 12-homoerythromycin A, 10-homoerythromycin A, 8-
epihomoerythromycin A, 6-epihomoerythromycin A, 4-

3 0 epihomoerythromycin A and 2-homoerythromycin A, respectively, all of
which are included within the scope of the present invention. Similarly,
introduction of acyltransferase segments carrying desired specificities for
the starter or extender unit into *eryA* DNA that results in the synthesis of
novel compounds are included within the scope of the present invention.

3 5 The erythromycin analogs produced by the method of this invention are
structurally similar to known antibacterial and prokinetic agents.
       It will also occur to those skilled in the art that genetic
manipulations described herein need not be limited to *Sac. erythraea*.

36

Suitable hosts are any other polyketide-producing <u>Actinomyces</u> where
DNA can be precisely inserted into the chromosome. Hence, the choice of
a convenient host is based solely on the relatedness of the novel
polyketide to a natural counterpart so as to minimize the number of

5   module rearrangements required for its biosynthesis. Therefore, Type I,
Type II and Type III alterations can be constructed in other <u>Actinomyces</u>
employing either endogenous or exogenous modules to produce novel
polyketides employing strategies analogous to those described herein for
Sac. erythraea. Thus all Type I, Type II or Type III mutations or various

10 combinations thereof constructed in other actinomycetes according to the
principles described herein, and the respective polyketides produced from
such strains, are included within the scope of the present invention.
Examples of polyketides that can be altered by creating Type I, Type II or
Type III changes in the producing microorganisms include, but are not

15 limited to macrolide antibiotics such as erythromycin, tylosin, spiramycin,
etc.; ansamacrolides such as rifamycins, maytansines, etc.; polyketide
antibiotics such as tetracycline; polyethers such as monesin, salinomycin,
etc.; polyenes such as candicidin, amphothericins; immunosuppressants
such as FK506, ascomycin, rapamycin, etc. and other complex polyketides

20 such as avermectin.
        Whereas the novel derivatives or modifications of erythromycin
described herein have been specified as the A derivatives, such as 7-
hydroxyerythromycin A, 11-oxo-11-deoxyerythromycin A, 14[1(1-
hydroxypropyl)]erythromycin A, etc., those skilled in the art understand

25 that the wild type strain of *Sac. erythraea* produces a family of
erythromycin compounds, including erythromycin A, erythromycin B,
erythromycin C and erythromycin D. Thus, modified strains of *Sac.
erythraea*, such as strain AKR2, for example, would be expected to produce
the corresponding members of the 11-oxo-11-deoxyerythromycin family,

30 including 11-oxo-11-deoxyerythromycin A, 11-oxo-11-deoxyerythromycin
B, 11-oxo-11-deoxyerythromycin C, and 11-oxo-11-deoxyerythromycin D.
Similarly, strain AM412 would be expected to produce not only 14(1-
propyl)erythromycin A but also the other members of the 14(1-
propyl)erythromycin family including 14(1-propyl)erythromycin B, 14(1-

35 propyl)erythromycin C and 14(1-propyl)erythromycin D. Similarly, all
other modified strains of *Sac. erythraea* described herein that produce
novel erythromycin derivatives would be expected to produce the A, B, C,
and D forms of said derivatives. Therefore, all members of the family of

each of the novel polyketides described herein are included within the scope of the present invention.

Variations and modifications of the methods for obtaining the desired plasmids, hosts for cloning and choices of vectors and segments of eryA DNA to clone and modify, other than those described herein that result in substantially the same strains and same products as those described herein will occur to those skilled in the art. For example, although we have described the use of the plasmids pWH3 and pWHM4 as E. coli-Sac. erythraea shuttle vectors, other vectors can be employed wherein all or part of pWHM3 or pWHM4 is replaced by other DNA segments that function in a similar manner, such as replacing the pUC19 component of pWHM3 and pWHM4 with pBR322, available from BRL, employing different segments of the pIJ101 or pJV1 replicons in pWHM3 and pWHM4, respectively, or employing selectable markers other than thiostrepton- and ampicillin-resistance. These are just few of a long list of possible examples all of which are included within the scope of the present invention. Similarly, the segments of the eryA locus subcloned into pWHM3 for generating strains AKS1, AKS2, etc. specified herein can readily be substituted for other segments of different length encoding the same functions, either produced by PCR-amplification of genomic DNA or of an isolated clone, or by isolating suitable restriction fragments from Sac. erythraea. In the same way, it is possible to create eryA strains carrying mutations functionally equivalent to those described herein by deleting different portions of the corresponding genes, by creating insertions into them, or by site-directed mutagenesis of specific nucleotide residues. Moreover, Sac. erythraea strains with mutant alleles other than the β-ketoacyl ACP synthase portions of eryA can be employed as hosts for gene conversion; Type III mutants can be constructed by double reciprocal crossover as exemplified for Type I and Type II mutants rather than by the gene conversion method described herein. Additional modifications include changes in the restriction sites used for cloning or in the general methodologies described above. All such changes are included in the scope of the invention. It will also occur to those skilled in the art that different methods are available to ferment Sac. erythraea, to extract the novel polyketides specified herein, and to synthesize substrate analogs, and that all such methods are also included within the scope of the present invention.

It will be apparent that many modifications and variations of the invention as set forth herein are possible without departing from the spirit and scope thereof, and that, accordingly, such limitations are imposed only as indicated by the appended claims.

5

What is claimed is:

1.   A method for directing the biosynthesis of specific polyketide
analogs by genetic manipulation of a polyketide-producing
5   microorganism, said method comprising the steps of:
         (1) isolating a polyketide biosynthetic gene-containing DNA
sequence;
         (2) identifying enzymatic activities associated within said gene-
containing DNA sequence;
10   (3) introducing one or more specified changes into said gene-
containing DNA sequence which codes for one of said enzymatic activities
resulting in an altered DNA sequence;
         (4) introducing said altered DNA sequence into a polyketide-
producing microorganism to replace the original sequence;
15   (5) growing a culture of the altered microorganism under
conditions suitable for the formation of the specific polyketide analog; and
         (6) isolating said specific polyketide analog from the culture.

2.   The method of claim 1 wherein said polyketide biosynthetic gene-
20   containing DNA sequence comprises genes which encode the enzymatic
activities comprising a polyketide synthase.

3.   The method of claim 2, wherein said polyketide synthase enzymatic
activities comprise β-ketoreductase, dehydratase, acyl carrier protein,
25   enoylreductase, β-ketoacyl ACP synthase, and acyltransferase.

4.   The method of claim 1 wherein said alteration which occurs in the
DNA sequence results in the inactivation of one or more enzymatic
activities involved in the processing of the β-carbonyl of said polyketide.
30

5.   The method of claim 4, wherein said inactivated enzymatic
activities affecting the processing of the β-carbonyl of said polyketide
comprise β-ketoreductase, dehydratase, and enoylreductase.

35 6.   The method of claim 4 wherein said alteration in the DNA
sequence results in the addition of one or more enzymatic activities
involved in the β-carbonyl processing of said polyketide.

40

7.   The method of claim 6 wherein said additional enzymatic activities are selected from the group consisting of β-ketoreductase, β-ketoreductase and dehydratase, and β-ketoreductase, dehydratase and enoylreductase.

8.   The method of claim 1 wherein said alteration occurring in the DNA segment results in the inactivation of one or more enzymatic activities involved in the condensation of carbon units to the nascent polyketide structure.

9.   The method of claim 8 wherein said enzymatic activities affecting the condensation of carbon units to the nascent polyketide structure comprise β-ketoacyl ACP synthase, acyl carrier protein and acyltransferase.

10.   The method of claim 1 wherein said alteration in the DNA sequence results in the change of the length of the polyketide synthesized.

11.   The method of claim 10 wherein said alteration results in the increase of the length of the polyketide.

12.   The method of claim 11 wherein said alteration comprises the addition of DNA sequences encoding the enzymatic activities consisting of acyltransferase, acyl carrier protein and β-ketoacyl ACP synthase.

13.   The method of claim 10 wherein said alteration results in the decrease of the length of the polyketide.

14.   The method of claim 13 wherein said alteration consists of the deletion of a DNA segment between two sequences encoding corresponding enzymatic activities.

15.   The method of claim 14 wherein said corresponding enzymatic activities are selected from the group consisting of β-ketoreductases, dehydratases, acyl carrier proteins, enoylreductases, β-ketoacyl ACP synthases, and acyltransferases.

16.   The method of claim 1 wherein said alteration consists in the replacement of the DNA segment encoding an acyltransferase with a DNA segment encoding an acyltransferase of different specificity.

41

17.   The method of claim 1 wherein said DNA sequence is isolated from a species from the *Actinomycetales* family.

5   18.   The method of claim 17 wherein said DNA sequence is isolated from a genus selected from the group consisting of *Actinomyces*, *Dactylosporangium*, *Micromonospora*, *Nocardia*, *Sac.*, *Streptoverticillium*, and *Streptomyces*.

10   19.   The method of claim 17 wherein said genus is selected from the group consisting of *Saccharapolyspora* and *Streptomyces*.

20.   The method of claim 19 wherein said genus is *Saccharapolyspora* and the species is *erythraea*.

15

21.   The method of claim 19 wherein said genus is *Streptomyces* and the species is *hygroscopicus*.

22.   The method of claim 1 wherein said polyketide is selected from the
20   group consisting of macrolides, tetracyclines, polyethers, polyenes, ansaymcins and derivatives or analogs thereof.

23.   The method of claim 22 wherein said polyketide is a macrolide.

25   24.   The method of claim 23 wherein said macrolide is an erythromycin.

25.   The method of claim 24 wherein said erythromycin analog is selected from the group consisting of 11-oxo-11-deoxyerythromycin A, 7-hydroxyerythromycin A, 6-deoxy-7-hydroxyerythromycin A, 7-
30   oxoerythromycin A, 3-oxo-3-deoxy-5-desosaminylerythronolide A, Δ-6,7-anhydroerythromycin A, ((14S,15S)14(1-hydroxyethyl)erythromycin A, 11-epifluoro-15-norerythromycinA, 14-(1-propyl)erythromycin A, 14(1-propyl)erythromycin A, and 14[1(1-hydroxypropyl))]erythromycin A.

35   26.   The method of claim 1 wherein said DNA sequence, designated *eryA*, encodes the enzymatic activities associated with the formation of 6-deoxyerythronolide B.

27. The method of claim 26 wherein said DNA sequence comprises: the DNA sequence of Figure 2.

28. The method of claim 1 wherein said gene-containing DNA
5 sequence encodes one or more enzymatic activities in the rapamycin biosynthetic pathway.

29. The method of claim 23 wherein said macrolide is a rapamycin analog.
10

30. A compound selected from the group consisting of 7-hydroxyerythromycin A; 6-deoxy-7-hydroxyerythromycin A; 7-oxoerythromycin A, 3-oxo-3-deoxy-5-desosaminyl-erythronolide A; Δ-6,7-anhydroerythromycin A; ((14S,15S)14(1-hydroxyethyl)erythromycin A; 11-
15 epifluoro-15-norerythromycin A; 14-(1-propyl)erythromycin A; 14(1-propyl)erythromycin A; and 14[1(1-hydroxypropyl)]erythromycin A.

FIG.1

FIG. 2—1
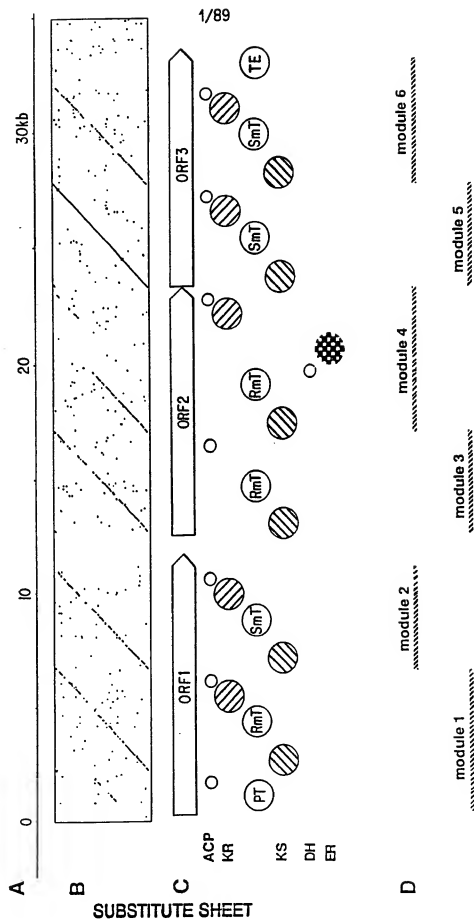
```
              L  L  G  E  S  R  V  F  A  A  A  M  D  A  C  A  R  A  F  E  -
     AGCCGGTGACCGACTGGACGCTGGGCAGGTTCTGGACTCTCCCGAGCAGTCGCCGCCG
1021 ----+----+----+----+----+----+----+----+----+----+----+  1080

              P  V  T  D  W  T  L  A  Q  V  L  D  S  P  E  Q  S  R  R  V  -
     TCGAGGTCGTCCAGCCCGCCCTGTTCGCGGTGCAGACGTCGCTGGCCGGCCGCTCTGGCGCT
1081 ----+----+----+----+----+----+----+----+----+----+----+  1140

              E  V  V  Q  P  A  L  F  A  V  Q  T  S  L  A  A  L  W  R  S  -
     CCTTCGGGCGTGACCCCCGACGCCGTGGTGGGCCACAGCATCGGCGAGCTGGCCGCGCGCGC
1141 ----+----+----+----+----+----+----+----+----+----+----+  1200

              F  G  V  T  P  D  A  V  V  G  H  S  I  G  E  L  A  A  A  H  -
     ACGTGTGCGGTGCGGGTGCCGCCGCACGCCGCCGCCGCCGCGCCGGCTGTGGAGCCGCCG
1201 ----+----+----+----+----+----+----+----+----+----+----+  1260

              V  C  G  A  A  A  D  A  A  R  A  A  A  L  W  S  R  E  -
     AGATGATTCCGTTGGTGGGCAACGGCGACATGGCAGCCGTCGCCGCTCTCCGCCGACGAGA
1261 ----+----+----+----+----+----+----+----+----+----+----+  1320

              M  I  P  L  V  G  N  G  D  M  A  A  V  A  L  S  A  D  E  I  -
     TCGAGCCCGCCATCGCCCCGGTGGGACGACGGTGTGCTGGCCGGGGTCAACGGTCCGC
1321 ----+----+----+----+----+----+----+----+----+----+----+  1380
```

```
        E   P   R   I   A   R   W   D   D   D   V   V   L   A   G   V   N   G   P   R
1381    GCTCGGTTCTGCTGACCGGTCGCCGGGAACCGGTCGCCGGCCCGGTCCCGGTCCAGGAGCTCTCGG
        ----+----+----+----+----+----+    1440

        S   V   L   L   T   G   S   P   E   P   V   A   R   R   V   Q   E   L   S   A
1441    CCGAGGGGGTCCGCCCACAGGTCATCAATGTGTCGATGGCGGCCGCCACTCGGCGCAGGTCG
        ----+----+----+----+----+----+    1500

        E   G   V   R   A   Q   V   I   N   V   S   M   A   A   H   S   A   Q   V   D
1501    ACGACATCGCCGAGGGGATGCGCTCGGCCCTGGCGTGGTTCGCCCCCGTGGCTCGGGAGG
        ----+----+----+----+----+----+    1560

        D   I   A   E   G   M   R   S   A   L   A   W   F   A   P   G   G   S   E   V
1561    TGCCCTTCTACGCCCAGCCTCACCCGGAGGTGCGGTCGACACCGGGAGCTGGTGGCCGACT
        ----+----+----+----+----+----+    1620

        P   F   Y   A   S   L   T   G   G   A   V   D   T   R   E   L   V   A   D   Y
1621    ACTGGCGCCCGCAGCTTCCGGCTGCCGGTGCCGCTTCGACGAGGGCGATCCGGTCCGCCCTGG
        ----+----+----+----+----+----+    1680

        W   R   R   S   F   R   L   P   V   R   F   D   E   A   I   R   S   A   L   E
```

FIG.2-3

```
1681 AGGTCGGTCCGGCACGTTCGTCGAAGCGAGCCGCACCCGTGTCTGGCCGCCGGGCTCC 1740
     ---------+---------+---------+---------+---------+---------+
       V  G  P  G  T  F  V  E  A  S  P  H  P  V  L  A  A  A  L  Q  -

1741 AGCAGACGCTCGACGCCCAGGGCTCCTCGGCGGCGGTGGTCCCGACGCTGCAACGCGGGC 1800
     ---------+---------+---------+---------+---------+---------+
       Q  T  L  D  A  E  G  S  S  A  A  V  V  P  T  L  Q  R  G  Q  -

1801 AGGGCCGGCATGCGGCGGTTCCTGCTGGCCGCGGCCCAGGCGTTCACCGGCGGCGTGGCCG 1860
     ---------+---------+---------+---------+---------+---------+
       G  M  R  R  F  L  L  A  A  A  Q  A  F  T  G  G  V  -

1861 TCGACTGGACCGCCGCCTACGACGACGTGGGGCCGAACCCGGCTCTCTGCCGGAGTTCGC 1920
     ---------+---------+---------+---------+---------+---------+
       D  W  T  A  A  Y  D  D  D  V  G  P  N  P  A  L  C  R  S  S  R  -

1921 GCCGGCCGAGGAGAAGACGAGCCGGCCGAGTCCGGCGTCGACTGGAACGCGCCACCGCA 1980
     ---------+---------+---------+---------+---------+---------+
       R  P  R  K  T  S  R  P  S  P  A  S  T  G  T  R  H  R  T  -

1981 CGTGCTGCGAGCGGCTGCTCCGGGTCGTCAACGGCGAGACCGCGGCGTTGGCGGGCCCG 2040
     ---------+---------+---------+---------+---------+---------+
       C  E  R  L  L  A  V  V  N  G  E  T  A  A  L  A  G  R  E  -
```

FIG.2-4

```
2041  AAGCCGACGCCGAGGCCACGTTCCGCGAGCTGGGGCTGGACTCCGTGCCGCCGCAGC      2100
      ---------+---------+---------+---------+---------+---------+
       A   D   E   A   T   F   R   E   L   G   L   D   S   V   L   A   A   Q   L  -

2101  TGGCGGCCAAGGTGAGCGCCGCGATCGGGCGGGAGGTCAACATCGCCCTGCTCTACGACC      2160
      ---------+---------+---------+---------+---------+---------+
       R   A   K   V   S   A   A   I   G   R   E   V   N   I   A   L   L   Y   D   H  -

2161  ACCCGACTCCGCGTGCCCTCGCGGAAGCACTCGCGCGCGGGAACCGAGGTCGCACAACGGG      2220
      ---------+---------+---------+---------+---------+---------+
       P   T   P   R   A   L   A   E   A   L   A   A   G   T   E   V   A   Q   R   E  -

2221  AAACCCGCGCGGGACCAACGAAGCGGCGCCCGGCCGGCGTCGCGGTCGTCGCCGATGG      2280
      ---------+---------+---------+---------+---------+---------+
       T   R   A   R   T   N   E   A   A   P   G   E   P   V   A   V   V   A   M   A  -

2281  CCTGCCGGCTGCCCGGCGGTGTGAGCACCCCCGGAGGAGTTCTGGGAGCTGCTGTCGGAGG      2340
      ---------+---------+---------+---------+---------+---------+
       C   R   L   P   G   G   V   S   T   P   E   E   F   W   E   L   L   S   E   G  -

2341  GCCGCGACGGCGGTCGGGGGACTGCCGACACCGGCGGACCTGGGACCTCGCTGTTCC      2400
      ---------+---------+---------+---------+---------+---------+
```

FIG.2-5

```
          R   D   A   V   A   G   L   P   T   D   R   G   W   D   L   D   S   L   F   H  -
2401  ACCCCGACCCCACGCGCCTCGGGCACCGCCCACCAGCGCGGCGGCGGTTTCCTGACCGAGG
      ----+----+----+----+----+----+----+----+----+----+----+----+ 2460

          P   D   P   T   R   S   G   T   A   H   Q   R   G   G   G   G   F   L   T   E   A  -
2461  CGACCGCCGTTCGACCCGGCCTTCTCGCGCATGTCCCGCCGCGAGGCGCTGGCCGTCGACC
      ----+----+----+----+----+----+----+----+----+----+----+----+ 2520

          T   A   E   D   P   A   F   F   G   M   S   P   R   E   A   L   A   V   D   P  -
2521  CGCAGCAGCGGCTCATGCTCGAGCTCTCCTGGGAAGTGCTGGAACGGGCGGGAATCCCGC
      ----+----+----+----+----+----+----+----+----+----+----+----+ 2580

          Q   Q   R   L   M   L   E   L   S   W   E   V   L   E   R   A   G   I   P   P  -
2581  CGACCTCGTTGCAGGCCTCGCCCACTGGCGTGTTCGTCGGCCTGATCCCGCAGGAGTACG
      ----+----+----+----+----+----+----+----+----+----+----+----+ 2640

          T   S   L   Q   A   S   P   T   G   V   F   V   G   L   I   P   Q   E   Y   G  -
2641  GCCCGGCGGCTGGCCGAGGGCGGCGAAGGCGTCGAGGGCTACCTGATGACCGGTACGACCA
      ----+----+----+----+----+----+----+----+----+----+----+----+ 2700

          P   R   L   A   E   G   G   E   G   V   E   G   Y   L   M   T   G   T   T  -
2701  CGAGCGTGCCCTCCGGCCCATCGCCTCACACGCTCGGCCTGGAGGGCCCGGATCAGCG
      ----+----+----+----+----+----+----+----+----+----+----+----+ 2760
```

```
        S   V   A   S   G   R   I   A   Y   T   L   G   L   E   G   P   A   I   S   V   -
2761    TGGACACCCGTGCTCGTCTCCGGTCGGTGGTCGCCGGTGGACCTGGCGTGGCACCTGGCGTGGCAGTCGCTGCGGC
        ------+---------+---------+---------+---------+---------+    2820

        D   T   A   C   S   S   L   V   A   V   H   L   A   C   Q   S   L   R   R   -
2821    GCGGCGAGTCGTCGCTGGCGATGGCAGGCGGTGTCACGGTGATGCCGACGCCCGGCATGC
        ------+---------+---------+---------+---------+---------+    2880

        G   E   S   S   L   A   M   A   G   G   V   T   V   M   P   T   P   G   M   L   -
2881    TGGTGGACTTCAGCCGGATGAACTCGCTGGCGCCGGACGGCCGGTGCAAGGCTTTCCCG
        ------+---------+---------+---------+---------+---------+    2940

        V   D   F   S   R   M   N   S   L   A   P   D   G   R   C   K   A   F   S   A   -
2941    CCGGCGCCAACGGTTTCGGCATGGCCGAGGGCGCGGGGATGCTCCTGCTGGAGCGGCTTT
        ------+---------+---------+---------+---------+---------+    3000

        G   A   N   G   F   G   M   A   E   G   A   G   M   L   L   E   R   L   S   -
3001    CGGACGCCCGCCGCAACGGCCACCCGGTGCTCGCCGTGCTCAGGGGACGGCGGCGTCAACT
        ------+---------+---------+---------+---------+---------+    3060

        D   A   R   R   N   G   H   P   V   L   A   V   L   R   G   T   A   V   N   S   -
        CCGACGGCGGCGAGCAACGGCTGTTCGCCGCCCAACGCGGCGGCGCCAGGTGCGGGTCATCC
```

FIG.2-7

```
3061 ------+---------+---------+---------+---------+---------+ 3120
     AGCAGGGCGCTGGCAGAGTCCGTCTCGGCCCGCCGCCGACATCGACGCGCCTCGAGCGGCCACG
     D   G   A   S   N   G   L   S   A   P   N   G   R   A   Q   V   R   V   I   Q

3121 ------+---------+---------+---------+---------+---------+ 3180
     GACCGGTACCCGGACTCGGCGACCCGATCGAGGCGCGGCCGCTGTTCGAGGCGTACGGGC
     Q   A   L   A   E   S   G   L   G   P   A   D   I   D   A   V   E   A   H   G

3181 ------+---------+---------+---------+---------+---------+ 3240
     GGCACCCGAGCAGCCCCTGCACCTGGGCTCGGTCAAGTCCAACCTCGGCCACACCCAGG
     T   G   T   R   L   G   D   P   I   E   A   R   A   L   F   E   A   Y   G   R

3241 ------+---------+---------+---------+---------+---------+ 3300
     GCGACCCGGAGCAGCCGCTGCACCTGGGCTCGGTCAAGTCCAACCTCGGCCACACCCAGG
     D   R   E   Q   P   L   H   L   G   S   V   K   S   N   L   G   H   T   Q   A

3301 ------+---------+---------+---------+---------+---------+ 3360
     CGGCCGCCGGTGTTGCCGGCGTGATCAAGATGGTGCTGGCGATGCGCGGCGGGCACCCTTC
     A   A   G   V   A   G   V   I   K   M   V   L   A   M   R   A   G   T   L   P

3361 ------+---------+---------+---------+---------+---------+ 3420
     CCCGCCACTCTGCACGCATCGGAGCGGTCGAAGGAGATCGACTGGTCATCCGGTGCGATCA
     R   T   L   H   A   S   E   R   S   K   E   I   D   W   S   S   G   A   I   S
```

FIG.2-8

```
3421  GCCTGCTCGACGAGCCGGAGCCGTGGCCCGCCGGCGCGCCCGGGCGGGGTCT  3480
      ----+----+----+----+----+----+----+----+----+----+
       L  L  D  E  P  E  P  W  P  A  G  A  R  P  R  R  A  G  V  V  S

3481  CGTCGTTCGCATCAGCGGCGCACCAACGGCGCACGCCATCATCGAGGAAGCTCCGCAGGTCG  3540
      ----+----+----+----+----+----+----+----+----+----+
       S  F  G  I  S  G  T  N  A  H  A  I  I  E  E  A  P  Q  V  V

3541  TCGAAGGCGAGCGGGTCGAGGCCCGGCGACGTCGTCGGCGCCCTGGGTGCTTTCGGCGAGCA  3600
      ----+----+----+----+----+----+----+----+----+----+
       E  G  E  R  V  E  A  G  D  V  V  A  P  W  V  L  S  A  S  S

3601  GCGCGGAAGGTCTGCGCGGCGCCAGGCGGCGGCTGGCCCGGCGCACCTGCGCGAGCACCCCG  3660
      ----+----+----+----+----+----+----+----+----+----+
       A  E  G  L  R  A  Q  A  A  R  L  A  A  H  L  R  E  H  P  G

3661  GTCAGGACCCGCGCGACATCGCGTACTCGCTCCGCGACGGGACCGGCCGCTGCCCCACC  3720
      ----+----+----+----+----+----+----+----+----+----+
       Q  D  P  R  D  I  A  Y  S  L  A  T  G  R  A  A  L  P  H  R

3721  GCGCCCGCCTTCGCCCCGTCGACGAGTCCGCGGCGCTGCGCGTGCTCGACGGTCTCGGA  3780
      ----+----+----+----+----+----+----+----+----+----+
       A  A  F  A  P  V  D  E  S  A  A  L  R  V  L  D  G  L  A  T
```

FIG.2-9

```
3781 CGGGAAACGCCACGGTGCCGCCGTTGGAACGAGCGGGCGCAGCGAGCGCCGTCTTCG
     ----+----+----+----+----+----+----+----+----+----+----+ 3840
      G  N  A  D  G  A  A  V  G  T  S  R  A  Q  Q  R  A  V  F  V -

3841 TCTTCCCCGGGCAGGGTTGGCAGTGGCGGCATGGCCGTCGACCTGCTCGACACCTCCC
     ----+----+----+----+----+----+----+----+----+----+----+ 3900
      F  P  G  Q  G  W  Q  W  A  G  M  A  V  D  L  L  D  T  S  P -

3901 CGGTTTCGCAGCCGCGTTGCGCGAGTGCCGACGGCGCTCGAACCGCATCTGGACTTCG
     ----+----+----+----+----+----+----+----+----+----+----+ 3960
      V  F  A  A  A  L  R  E  C  A  D  A  L  E  P  H  L  D  F  E -

3961 AGGTGATCCCGTTCCTGCGCGGAAGCCCGCGAGGCGGGAGCAGGAGCGCGGCGTGTCGA
     ----+----+----+----+----+----+----+----+----+----+----+ 4020
      V  I  P  F  L  R  A  E  A  A  R  R  E  Q  D  A  A  L  S  T -

4021 CCGAGCGCGTGGACGTGGTGCAGCCCGTGATGTTCGCGGTCATGGTCTCCGGCGTCGA
     ----+----+----+----+----+----+----+----+----+----+----+ 4080
      E  R  V  D  V  V  Q  P  V  M  F  A  V  M  V  S  L  A  S  M -

4081 TGTTGGCGAGCCCACGGCGTCGAGCCCGCGGGCACTCCCAGGGGCGAGATCG
     ----+----+----+----+----+----+----+----+----+----+----+ 4140
      W  R  A  H  G  V  E  P  A  A  V  I  G  H  S  Q  G  E  I  A -
```

FIG.2-10

```
4141 CCGCCGCTGCGTCGGGGGCGGCGCTCTCGCTGTGGACGACGCCGCGGCCGCTGGTCGGCGGCTGC +4200
     -----+---------+---------+---------+---------+---------+
      A  A  C  V  A  G  A  L  S  L  D  D  A  A  R  V  V  A  L  R -

4201 GCAGCCGCGGTCATCGCCACCATGCCCGGGAACAAGGGCATGGCCTCGATCGCCGCTCCGG +4260
     -----+---------+---------+---------+---------+---------+
      S  R  V  I  A  T  M  P  G  N  K  G  M  A  S  I  A  A  P  A -

4261 CCGGGGAAGTCCGGGCGCGGAATCGGTGACCGCGTCGAGATCGCCGCCGTCAACGGTCCGC 4320
     -----+---------+---------+---------+---------+---------+
      G  E  V  R  A  R  I  G  D  R  V  E  I  A  A  V  N  G  P  R -

4321 GCTCGGTGGTGGTCGCCGGCGACAGCGAACTGGACCGGCTGGTCGCTTCCTGCACCA +4380
     -----+---------+---------+---------+---------+---------+
      S  V  V  A  G  D  S  E  L  D  R  L  V  A  S  C  T  T -

4381 CCGAGTGCATCCGCGCCAAGCGGCTGGCCGTGGACTACGGCGTCGCCACTCCTCGCACGTCG +4440
     -----+---------+---------+---------+---------+---------+
      E  C  I  R  A  K  R  L  A  V  D  Y  A  S  H  S  S  H  V  E -

4441 AGACGATCCGAGACGCCACTGCACGCCGAGCTGGGAGAGAGGACTTCCACCCGCTGCCGGGGT +4500
     -----+---------+---------+---------+---------+---------+
```

```
          T   I   R   D   A   L   H   A   E   L   G   E   D   F   H   P   L   P   G   F  -
      TCGTGCCCTTCTTCTCCACCGTCACCGGGCGGCTGGACGGCGAGCCGGACGAGCTCGACGCCG
4501  ---------+---------+---------+---------+---------+---------+  4560

          V   P   F   F   S   T   V   T   G   R   W   T   Q   P   D   E   L   D   A   G  -
      GGTACTGGTACCGGAACCTGCGCGACCGTGCGGTTCGGCGACGCCGTCCGTGCGCTCG
4561  ---------+---------+---------+---------+---------+---------+  4620

          Y   W   Y   R   N   L   R   R   T   V   R   F   A   D   A   V   R   A   L   A  -
      CCGAGCAGGGATATCGCACGTTCCTGGAGGTCGAGGTCAGCCGCGACCGGATCCTCACCGCCGCGA
4621  ---------+---------+---------+---------+---------+---------+  4680

          E   Q   G   Y   R   T   F   L   E   V   S   A   H   P   I   L   T   A   A   I  -
      TCGAGGAGATCGGCGACGGATCGGGCCGCGACCTCTCCGCCATCCATTCGCTGCCGCCGCG
4681  ---------+---------+---------+---------+---------+---------+  4740

          E   E   I   G   D   G   S   G   A   D   L   S   A   I   H   S   L   R   R   G  -
      GTGACGGCAGCCTCGCGGACTTCGGCGAAGCCCTCTCCGCGCGTTCGCCGCGGTGTCG
4741  ---------+---------+---------+---------+---------+---------+  4800

          D   G   S   L   A   D   F   G   E   A   L   S   R   A   F   A   A   G   V   A  -
      CGGTGGACTGGGAGTCGGTGCACCTGGGCACCGGAGCACGGCCGGGGTGCCCTTGCCCACCT
4801  ---------+---------+---------+---------+---------+---------+  4860
```

FIG.2-11

FIG.2-12

```
     V  D  W  E  S  V  H  L  G  T  G  A  R  R  V  P  L  P  T  Y
4861 ACCCGTTCCAGCGCGAGCGCGTCTGGCTCGAACCGGAGCCGGCGGCCGTCCACCG 4920
     --------+---------+---------+---------+---------+---------+

     P  F  Q  R  E  R  V  W  L  E  P  K  P  V  A  R  R  S  T  E
4921 AGGTCGACGAGGTTTCCGCGCTGCCTACCGCATCGAGTGGCGGCCCACCGGTGCCGGGTG 4980
     --------+---------+---------+---------+---------+---------+

     V  D  E  V  S  A  L  R  Y  R  I  E  W  R  P  T  G  A  G  E
4981 AACCCGCCCGGCTCGACGGCACCTGGCTGGTGGCGAAGTACGCCGGAACCCGGACGAGA 5040
     --------+---------+---------+---------+---------+---------+

     P  A  R  L  D  G  T  W  L  V  A  K  Y  A  G  T  A  D  E  T
5041 CGAGCACCCGGCTCGGGAGGCCCTGGAGTCGGCGGGTCCGGGTCCGCGAACTGGTCG 5100
     --------+---------+---------+---------+---------+---------+

     S  T  A  A  R  E  A  L  E  S  A  G  A  R  V  R  E  L  V  V
5101 TGGACGCCCGCTGCGGGTCGGACGAACTCGGCGGCGGCCGGCTTCGTTCGGTCGGCGAGGTGG 5160
     --------+---------+---------+---------+---------+---------+

     D  A  R  C  G  R  D  E  L  A  E  R  L  R  S  V  G  E  V  A
```

FIG.2-13

```
5161 CAGGAGTGCTGTCCCTGCTCGCGGTGGACGAAGCGGAGGAGGCGCCGCTCGCGC
     -----+----+----+----+----+----+  5220
        G  V  L  S  L  L  A  V  D  E  A  E  E  A  P  L  A  L -

5221 TGGCTTCGCTGGCGACACGCTCAGCCTCGTGCAGGCGATGGTGTCGCCGAACTCGGAT
     -----+----+----+----+----+----+  5280
        A  S  L  A  D  T  L  S  L  V  Q  A  M  V  S  A  E  L  G  C -

5281 GTCCGCTGTGACGGTGACGGAAAGCGCCGTCGCGCGACGGGGCCGTTCGAACGGTCCGCA
     -----+----+----+----+----+----+  5340
        P  L  W  T  V  T  E  S  A  V  A  T  G  P  F  E  R  V  R  N -

5341 ACGCCGCCACGGCGCCCTGTGGGGCGTCGGGCGGGTCATCGCGGTCGGAGAACCCCGCCG
     -----+----+----+----+----+----+  5400
        A  A  H  G  A  L  W  G  V  G  R  V  I  A  L  E  N  P  A  V -

5401 TGTGGGGCGGCCTGGTCGACGTGCCCGCGGGGTCGGTCGGTCGCCGAGCTGGCCCGCACCTCG
     -----+----+----+----+----+----+  5460
        W  G  G  L  V  D  V  P  A  G  S  V  A  E  L  A  R  H  L  A -

5461 CGGCGGTCGTGTCCGGCGGCGCCGGTGAGGACCAGCTCGCGCGTGCGCGGCGACGGGGGTGT
     -----+----+----+----+----+----+  5520
        A  V  V  S  G  G  A  G  E  D  Q  L  A  L  R  A  D  G  V  Y -
```

FIG.2-14

```
5521 ACGGACGCCGGTGGTGCCGCGCGGCCCGCGGCCGGACGAGTGAAAACCCACCG 5580
     -----+---------+---------+---------+---------+---------+ 
      G   R   R   W   V   R   A   A   A   P   A   T   D   D   E   W   K   P   T   G  -

5581 GAACCGTGCTGGTCACCGGTGGCACGGGCGGTGTCGGCGGCCAGATCGCGCGCTGGCTCG 5640
     -----+---------+---------+---------+---------+---------+ 
      T   V   L   V   T   G   G   T   G   G   V   G   G   Q   I   A   R   W   L   A  -

5641 CCCGGCCGGGGCGCGCCCCACCTGCTGCTGGTGAGCCGCTCAGCGGGCCCAGCGGCCGGACGGCG 5700
     -----+---------+---------+---------+---------+---------+ 
      R   R   G   A   P   H   L   L   L   V   S   R   S   G   P   D   A   D   G   A  -

5701 CCGGGCGAACTGGTCGCCGAGCTCGGAGGCGCCTGGGCGCCCGGACGACCGTCGCGGCCTGCG 5760
     -----+---------+---------+---------+---------+---------+ 
      G   E   L   V   A   E   L   E   A  .  L   G   A   R   T   T   V   A   A   C   D  -

5761 ACGTGACCGACCGCGAGTCCGGAGCTGCTCGGCGGCCATCGGTGACGACGTCCCGC 5820
     -----+---------+---------+---------+---------+---------+ 
      V   T   D   R   E   S   V   R   E   L   L   G   G   I   G   D   D   V   P   L  -

5821 TCTCGGGGGTGTTCACGCCCGCCGCCGCCACGCTCGACGACGGCACCGTGGACACCCTCACCG 5880
     -----+---------+---------+---------+---------+---------+ 
```

FIG.2-15

```
      S   A   V   F   H   A   A   A   T   L   D   D   G   T   V   D   T   L   T   G  L -
      GCGAGCGCATCGAGCGGGCAAGTCGCGCCAAGGTGCTCGGCGGCCAACCTGCACGAGC
5881  --------+---------+---------+---------+---------+---------+  5940

      E   R   I   E   R   A   S   R   A   K   V   L   G   A   R   N   L   H   E   L  L -
      TGACGCGCCAGCTGACCTGACCGCCTTCGTGCTGTTCTCGTCCTTCGGCCTTCG
5941  --------+---------+---------+---------+---------+---------+  6000

      T   R   E   L   D   L   T   A   F   V   L   F   S   S   F   A   S   A   F   G  G -
      GCGCCCCGGGCTCGGCCGCTACGGCCCGGCAACCGCCTACCTCGACGGCCTCGCCCAGC
6001  --------+---------+---------+---------+---------+---------+  6060

      A   P   G   L   G   G   Y   A   P   G   N   A   Y   L   D   G   L   A   Q   Q  Q -
      AGCGGCGGAGCGACGGACTCCCCGCCGTGGGGGACCTGGGCGGGCCAGCCG
6061  --------+---------+---------+---------+---------+---------+  6120

      R   R   S   D   G   L   P   A   T   A   V   A   W   G   T   W   A   G   S   G  G -
      GGATGGCCGAAGGCCGGTGGCCGACCCGTTCCGCAGGCACGGCGTCATCGAGATGCCTC
6121  --------+---------+---------+---------+---------+---------+  6180

      M   A   E   G   A   V   A   D   R   F   R   R   H   G   V   I   E   M   P   P  P -
      CCGAGACGGCGTCGCCGGCGTTGGCAGACCGGCGCGGCTGGAAGTCTCGCCCGATCG
6181  --------+---------+---------+---------+---------+---------+  6240
```

FIG. 2-16

```
            E   T   A   C   R   A   L   Q   N   A   L   D   R   A   E   V   C   P   I   V   -
6241   TCATCGACGTCAGGTGGGACCGGTTCCTGCTCGCCTACACCGGCCAGCCCGACCAGC   6300
       --------+---------+---------+---------+---------+---------+

            I   D   V   R   W   D   R   F   L   L   A   Y   T   A   Q   R   P   T   R   L   -
6301   TCCTTCGACGAGATCGACGACGCGGCGGGGCTGCGCGCCAGGCGCGGCGAACCGCGGG   6360
       --------+---------+---------+---------+---------+---------+

            F   D   E   I   D   D   A   R   R   A   A   P   Q   A   P   A   E   P   R   V   -
6361   TGGGCGCCGCTGGCCTCGCCCCGCGAGGCCGGAGGAAGCCGTGTTCGAGCTCGTGC   6420
       --------+---------+---------+---------+---------+---------+

            G   A   L   A   S   L   P   A   P   E   R   E   E   A   L   F   E   L   V   R   -
6421   GCTGCCACGGCGGCCGCCGTCCTCGGCCACGCCTCGGCGGGTGCCCCGCCGACCAGG   6480
       --------+---------+---------+---------+---------+---------+

            S   H   A   A   V   L   G   H   A   S   A   E   R   V   P   A   D   Q   A   -
6481   CCTTCGGGAACTCGGCCGTCGACTCGCTGTCGCGGCTTGAGCTGCGCCAACCGGCTCGGCG   6540
       --------+---------+---------+---------+---------+---------+

            F   A   E   L   G   V   D   S   L   S   A   L   E   L   R   N   R   L   G   A   -
       CCGGCACCGGTGTCCGCCTGCCGACGACGACCGTCTTCGACACCCCGACGTGCGGACGC
```

FIG. 2-17

```
6541 ---+---+---+---+---+---+---+---+---+---+---+---+ 6600
          A   T   G   V   R   L   P   T   T   V   F   D   H   P   D   V   R   T   L
     TGGCGGCGCACCTGGCCCGGCCGAACTCGGCGGTGGACGGGAGCCGGAGCCAGGGCGGCCACCGG

6601 ---+---+---+---+---+---+---+---+---+---+---+---+ 6660
          A   A   H   L   A   A   E   L   G   G   A   T   G   A   E   Q   A   A   P   A
     CGACCACGGCCCCGTCGACGAGCCGATCGCCGATCGTCGGCGATGGCGTGCCGGCTGCCCG

6661 ---+---+---+---+---+---+---+---+---+---+---+---+ 6720
          T   T   A   P   V   D   E   P   I   A   I   V   G   M   A   C   R   L   P   G
     GGGAGGTCGACTCCCCGGAGCGGCTGTGGGAGCTGATCACCTCGGGACGCGACTCCCGGG

6721 ---+---+---+---+---+---+---+---+---+---+---+---+ 6780
          E   V   D   S   P   E   R   L   W   E   L   I   T   S   G   R   D   S   A   A
     CGGAGGTCCCCGATCAGACCGGGGCTGGGTCCCCGACGAGCCTGATGGCCTCGACGCGGCGG

6781 ---+---+---+---+---+---+---+---+---+---+---+---+ 6840
          E   V   P   D   D   R   G   W   V   P   D   E   L   M   A   S   D   A   A   G
     GAACCCGCGCCACGGCAACTTCATGCGGCCGCGGTGACTTCGACGCGGCGGTTCTTCG

6841 ---+---+---+---+---+---+---+---+---+---+---+---+ 6900
          T   R   A   H   G   N   F   M   A   G   A   G   D   F   D   A   A   F   F   G
```

FIG.2-18

```
6901 GGATCTCGCCGCGGAGGCGCTGGCGATGGACCCGCAGCAGCGCCAGGCGCTGGAGACGA 6960
     ----+----+----+----+----+----+----+----+----+----+----+ -
       I  S  P  R  E  A  L  A  M  D  P  Q  Q  R  Q  A  L  E  T  T

6961 CGTGGGAGGCGCTGGAAAGCGCGGGCATCCCACCGGAGACGTTGCGGGCAGCGACACCG 7020
     ----+----+----+----+----+----+----+----+----+----+----+ -
       W  E  A  L  E  S  A  G  I  P  P  E  T  L  R  G  S  D  T  G

7021 GCGTGTTCGTCGGCATGTCCCACCAGGGCTACGGCGACCGGCGTCCGCGCCCGGAGGACG 7080
     ----+----+----+----+----+----+----+----+----+----+----+ -
       V  F  V  G  M  S  H  Q  G  Y  A  T  G  R  P  R  P  E  D  G

7081 GCGTCGACGGGTACCTGCTCACCGGCAACACCGCGAGCGTCCGCGTCCCGGGACCCATCGCCT 7140
     ----+----+----+----+----+----+----+----+----+----+----+ -
       V  D  G  Y  L  L  T  G  N  T  A  S  V  A  S  G  R  I  A  Y

7141 ACGTGCTGGGGCTGGAAGGTCCCGGCGCTGACGGTGGACACGGCGTGTTCGTCGTCGTTGG 7200
     ----+----+----+----+----+----+----+----+----+----+----+ -
       V  L  G  E  G  P  A  L  T  V  D  T  A  C  S  S  L  V

7201 TGGCGTTGCACACGGCGTGTGGGTCGTTGCGTGACGGTGACTGCGGTCTTGCGGTGGCCG 7260
     ----+----+----+----+----+----+----+----+----+----+----+ -
       A  L  H  T  A  C  G  S  L  R  D  G  D  C  G  L  A  V  A  G
```

FIG.2-19

```
7261 GTGGTGTCGGTGATGGCGGGTCCGGAGGTGTTCACCGAGTTCTCCGCCAGGGCCGGC
     ---------+---------+---------+---------+---------+---------+ 7320
      G  V  S  V  M  A  G  P  E  V  F  T  E  F  S  R  Q  G  A  L  -

7321 TCTCGCCGGACAGGCCGGTGCAAGCCCTTCTCGGACGAGGCCGACGATTCGGTCTCGGGG
     ---------+---------+---------+---------+---------+---------+ 7380
      S  P  D  G  R  C  K  P  F  S  D  E  A  D  G  F  G  L  G  E  -

7381 AGGGTTCGGCGTTCGTCGTGCTCCAGCGGTTGTCCGACGCCAGGCGGGAGGGCCGCCGCG
     ---------+---------+---------+---------+---------+---------+ 7440
      G  S  A  F  V  V  L  Q  R  L  S  D  A  R  R  E  G  R  R  V  -

7441 TGCTCGGCCGGTGGTGGCCGGTCCGGCGGTGAACCAGGACGGCGGAGCAACGGGCTCTCCG
     ---------+---------+---------+---------+---------+---------+ 7500
      L  G  V  V  A  G  S  A  V  N  Q  D  G  A  S  N  G  L  S  A  -

7501 CTCCGAGCCGCGTCGCGCAGCAGCGGGTCATCCGCCGGCGTGGGGCGTGCGGGGATCA
     ---------+---------+---------+---------+---------+---------+ 7560
      P  S  G  V  A  Q  Q  R  V  I  R  R  A  W  A  R  A  G  I  T  -

7561 CGGGCGGGATGTGGCCGTGGTGGAGGCCATGGACCGGCTACGCGCGGTGGGCGATCCGG
     ---------+---------+---------+---------+---------+---------+ 7620
      G  A  D  V  A  V  V  E  A  H  G  T  R  L  G  D  P  V  -
```

FIG.2-20

```
     TGGAGGGCGTCGGCGTTGCTGGCTACTTACGGCAAGTCGCGCGGGGTCGTCGGGCCCGGTGC
7621 ------+---------+---------+---------+---------+---------+ 7680
     E  A  S  A  L  L  A  T  Y  G  K  S  R  G  S  S  G  P  V  L  -

     TGCTGGGTTCGGTGAAGTCGAACATCGGTCACGCGCAGGCGGCCGCGGGTGTCGCGGGCG
7681 ------+---------+---------+---------+---------+---------+ 7740
     L  G  S  V  K  S  N  I  G  H  A  Q  A  A  A  G  V  A  G  V  -

     TGATCAAGGTGCTGCTCGGCCTGGAACGCGGTGTGCCCCCGATGCTGTGCCGGGGCG
7741 ------+---------+---------+---------+---------+---------+ 7800
     I  K  V  L  L  G  L  E  R  G  V  V  P  P  M  L  C  R  G  E  -

     AGAGGTCGGCCCTCATCGACTGGTCCTCGGCGAGATCGAGCTCGCAGACGGCGTGCGGGG
7801 ------+---------+---------+---------+---------+---------+ 7860
     R  S  G  L  I  D  W  S  S  G  E  I  E  L  A  D  G  V  R  E  -

     AGTGGTCGCCGGCCGACGGGGTGCGCCGGGCAGGTGTGTCGGCCGTTCGGGGTGAGCG
7861 ------+---------+---------+---------+---------+---------+ 7920
     W  S  P  A  A  D  G  V  R  R  A  G  V  S  A  F  G  V  S  G  -

     GGACGAAGCGCCACGTGATCATCGCCGAGCCCGGAACCGGAGCCCGTGCCGCAACCGC
7921 ------+---------+---------+---------+---------+---------+ 7980
```

```
      T  N  A  H  V  I  I  A  E  P  P  E  P  E  P  V  P  Q  P  R  -
      GACGCCATGCTGCCCGCGACCGGGTGGTGGTCCGGTGGCGTGCTGCTGGCCAGGACCGGGGCCGG
7981  ---+----+----+----+----+----+  8040

      R  M  L  P  A  T  G  V  V  P  V  V  L  S  A  R  T  G  A  A  -
      CGTTGCGGGCGCCAGGCCGGCAGGCTCGCCGCCGCCACCTCGCCCGCGCATCCCGGGATCGGCAC
8041  ---+----+----+----+----+----+  8100

      L  R  A  Q  A  G  R  L  A  D  H  L  A  A  H  P  G  I  A  P  -
      CGGCCGACGTGAGCTGGACGATGGCCGGGCCCCAGCACTTCGAGGAGCGGGCCGCGGCG
8101  ---+----+----+----+----+----+  8160

      A  D  V  S  W  T  M  A  R  A  R  Q  H  F  E  E  R  A  A  V  -
      TGCTCGCCCGCCGACACCGGCCGTGCCGAGGCCGTTGCGGGGGCCGGTGGCGACGGCGGCGGG
8161  ---+----+----+----+----+----+  8220

      L  A  A  D  T  A  E  A  V  H  R  L  R  A  V  A  D  G  A  V  -
      TGGTTCCCGGTGTTGTCACCGGCCAGTGCCTTCCGACGGTGTTCAGTGTTCGTCTTCCCTG
8221  ---+----+----+----+----+----+  8280

      V  P  G  V  V  T  G  S  A  S  D  G  G  S  V  F  V  F  P  G  -
      GGCAGGGTGCCCAGTGGGAAGGCATGGGCCCGGGAGTTGTTGCCCGTTCCCGTCTTCGCCG
8281  ---+----+----+----+----+----+  8340
```

FIG. 2-21

FIG.2-22

```
       Q   G   A   Q   W   E   G   M   A   R   E   L   L   P   V   P   V   F   A   E   -
8341  AGTCGATCGCCGAGTGCGATGCCGTGTTGTCCGAGGTGGCCGGATTCTCGGTGTCCGAGG
      ----+----+----+----+----+----+----+----+----+----+----+----+  8400

       S   I   A   E   C   D   A   V   L   S   E   V   A   G   F   S   V   S   E   V   -
8401  TGCTCGGAGCCAGTCCGGACGGCCCGTCCGTCGGTGAGCGGGTCGACGTGGTGCAGCCGGTGC
      ----+----+----+----+----+----+----+----+----+----+----+----+  8460

       L   E   P   R   P   D   A   P   S   L   E   R   V   D   V   V   Q   P   V   L   -
8461  TGTTCGCGGTTGATGTGTCGCTGGCGGCGGTTGTGGCGGTGCCCTGCCGTTCCTTCGG
      ----+----+----+----+----+----+----+----+----+----+----+----+  8520

       F   A   V   M   V   S   L   A   R   L   W   R   A   C   G   A   V   P   S   A   -
8521  CCGTCATAGGGCCACTCGCAGGGTGAGATCGCCCCGCGTGGTGGCGGGAGCGTTGTCGC
      ----+----+----+----+----+----+----+----+----+----+----+----+  8580

       V   I   G   H   S   Q   G   E   I   A   A   A   V   V   A   G   A   L   S   L   -
8581  TGGAGGACGGCATGCCGCGTCGTCGCCCCGGTCGAGGCGGTGCGGGTCGGGGGGGCC
      ----+----+----+----+----+----+----+----+----+----+----+----+  8640

       E   D   G   M   R   V   V   A   R   R   S   R   A   V   R   A   V   A   G   R   -
```

FIG.2-23

```
8641 GGGGAGCATGCTCTCGGTGCGCGGCGGGCCGCTCCGACGTCGAGAAGCTGCTCGCCGACG 8700
     ----+----+----+----+----+----+----+----+----+----+----+----+
      G   S   M   L   S   V   R   G   G   R   S   D   V   E   K   L   L   A   D   D

8701 ACAGCTGGACCGGCAGGCTGGAGGTCGCCGCGGTCAACGGCCCCGACGCCGTGGTGGTGG 8760
     ----+----+----+----+----+----+----+----+----+----+----+----+
      S   W   T   G   R   L   E   V   A   A   V   N   G   P   D   A   V   V   V   A

8761 CCGGTGACGCCCAGGCGGGCGAGTTCCTGGAGTACTGCGAGGGCGTGGGCATCCGCG 8820
     ----+----+----+----+----+----+----+----+----+----+----+----+
      G   D   A   Q   A   A   R   E   F   L   E   Y   C   E   G   V   G   I   R   A

8821 CCCGCGCCATCCCGGTGGACTACGCCTCGCACACCGCGCACGTCGAGCCCGTGCGCGACG 8880
     ----+----+----+----+----+----+----+----+----+----+----+----+
      R   A   I   P   V   D   Y   A   S   H   T   A   H   V   E   P   V   R   D   E

8881 AACTGGTCCAGGCGCTGGCCGGGATCACCCCGCGACGGCGCGAGGTGCCGTTCTTCTCCA 8940
     ----+----+----+----+----+----+----+----+----+----+----+----+
      L   V   Q   A   L   A   G   I   T   P   R   R   A   E   V   P   F   F   S   T

8941 CCCTGACCGGCGACTTCCTCGACGGCACCGAGCTGGACGCGGGCTACTGGTACCGCAACC 9000
     ----+----+----+----+----+----+----+----+----+----+----+----+
      L   T   G   D   F   L   D   G   T   E   L   D   A   G   Y   W   Y   R   N   L
```

FIG. 2-24

```
9001  TGGGTCACCCGGTGGAGTTCACTCCGCGTGCCAGGCGCTGACCGACCAGGGATACGCGA  9060
      ----+----+----+----+----+----+----+----+----+----+----+----+ -
      R   H   P   V   E   F   H   S   A   V   Q   A   L   T   D   Q   G   Y   A   T

9061  CGTTCATCGAGGTCAGCCCGCACCCGGTGCTGGCGTCGAGCGTCCAGGAGACCCTCGACG  9120
      ----+----+----+----+----+----+----+----+----+----+----+----+ -
      F   I   E   V   S   P   H   P   V   L   A   S   S   V   Q   E   T   L   D   D

9121  ACGCGGAGTCGGACGCGGCGGTGCTCGGACGCTGAACCGCACCGGGGCGACGCCGACC  9180
      ----+----+----+----+----+----+----+----+----+----+----+----+ -
      A   E   S   D   A   A   V   L   G   T   L   E   R   D   A   G   D   A   D   R

9181  GCTTCCTCACGGCCACTCGCCGACGCGCCACACGCGGTGTCGGCGGTCGACTGGGAAGCGG  9240
      ----+----+----+----+----+----+----+----+----+----+----+----+ -
      F   L   T   A   L   A   D   A   H   T   R   G   V   A   V   D   W   E   A   V

9241  TGCTCGGCCGGGCCGGACTGGTTCGACCTGCCGCGGGTTATCCTTTCCAGGGCAAGCGGTTCT  9300
      ----+----+----+----+----+----+----+----+----+----+----+----+ -
      L   G   R   A   G   L   V   D   L   P   G   Y   P   F   Q   G   K   R   F   W

9301  GGCTGCTCGCCGACCGGCACCACCCCTCGTGACGAGCGTCGACGGCTCGTGTTCTACCGGGTCG  9360
      ----+----+----+----+----+----+----+----+----+----+----+----+ -
```

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```
      L L P D R T T P R D E L D G W F Y R V D -
    ACTGGACCGAGGTGCCGGCGTTCCGAACCTGCCGCGGCCGCCGTTGGCTCGTGGTGG
9361 ---------+---------+---------+---------+---------+---------+ 9420

      W T E V P R S E P A A L R G R W L V V V -
    TGCCCGAGGGGCACGAGGAGGACGGCTGGACCGTGGAGGTGCGGTCCCGCCTCGCCCGAGG
9421 ---------+---------+---------+---------+---------+---------+ 9480

      P E G H E E D G W T V E V R S A L A E A -
    CCGGCGCCGAACCGAGGTCACGCGGCCGTCGGCGGGCTGGTCCGTGACTGCGCGGGGCCG
9481 ---------+---------+---------+---------+---------+---------+ 9540

      G A E P E V T R G V G G L V G D C A G V -
    TGGTGTCGTTGCTCGCCCTCGAGGGCGGATGGTGCGGTGCAAACCCTTGTCGTGGTGCGGG
9541 ---------+---------+---------+---------+---------+---------+ 9600

      V S L L A L E G D G A V Q T L V L V R E -
    AACTCGACGCCCAGGGGCATCGACGCGCCACTGTGGACGGTCACCTTCGGCGCGGTCGACG
9601 ---------+---------+---------+---------+---------+---------+ 9660

      L D A E G I D A P L W T V T F G A V D A -
    CGGGCAGTCGGTGGCCCGGACCAGGGAAGCTGTGGGGCGTGGGGCCAGGTCGCGT
9661 ---------+---------+---------+---------+---------+---------+ 9720
```

FIG. 2−25

```
            G  S  P  V  A  R  P  D  Q  A  K  L  W  G  L  G  Q  V  A  S -
      CCCTGGAACGCGGGGCCCGCTGGACCGGCCTCGTCGACCTGCCGCACATGCCGACCCGG
9721  --------+---------+---------+---------+---------+---------+  9780

            L  E  R  G  P  R  W  T  G  L  V  D  L  P  H  M  P  D  D  P  E -
      AACTGCCGAGGCCGTCTCACCCGGTGCTGGCCGCCGGAGGACCAGGTCGGCGGTGCGCG
9781  --------+---------+---------+---------+---------+---------+  9840

            L  R  G  R  L  T  A  V  L  A  G  S  E  D  Q  V  A  V  R  A -
      CCGACGCCGTGCGTGCGGCGGCTTCCCGCGCCACGTCACCGCCACCTCGGAGTACG
9841  --------+---------+---------+---------+---------+---------+  9900

            D  A  V  R  A  R  R  L  S  P  A  H  V  T  A  T  S  E  Y  A -
      CGGTGCCGGCCGGCCACAATCCTGGTCACCGGTGGCACCGCCGGCCTGGGCGCGGAGGTGG
9901  --------+---------+---------+---------+---------+---------+  9960

            V  P  G  G  T  I  L  V  T  G  G  T  A  G  L  G  A  E  V  A -
      CCCGGTGGCTCGCCGGTCGCGGCGGCCGAACACCTCGGCGTGGTCAGCAGGCAGGCCCGG
9961  --------+---------+---------+---------+---------+---------+  10020

            R  W  L  A  G  R  G  A  E  H  L  A  L  V  S  R  R  R  G  P  D -
      ACACCGGAGGGCGGTCGGCGACCTGACCCGCCGAGCTGACCCGGCTCGGCGGGGTGTCGG
```

FIG.2-26

```
10021 ---------+---------+---------+---------+---------+---------+ 10080
       T  E  G  V  G  D  L  T  A  E  L  T  R  L  G  A  R  V  S  V
      TGCACCGGTGCGACGTCAGCAGCCGCGAACCGGTGAGGGAACTCGTGCACGGCCTGATCG
10081 ---------+---------+---------+---------+---------+---------+ 10140
       H  A  C  D  V  S  S  R  E  P  V  R  E  L  V  H  G  L  I  E
      AGCAGGCGACGTCGTCCGCGGTGTGGTCACGCGCGGGACTGCCGCGCAGGTCGGCGA
10141 ---------+---------+---------+---------+---------+---------+ 10200
       Q  G  D  V  V  R  G  V  V  H  A  A  G  L  P  Q  Q  V  A  I
      TCAACGACATGGACGAGGCCGCCTTCGACGAGGTGGTCGCGGCCAAGGCCGGGGGCGGG
10201 ---------+---------+---------+---------+---------+---------+ 10260
       N  D  M  D  E  A  A  F  D  E  V  V  A  A  K  A  G  G  A  V
      TGCACCTGGACGAGCTGTGCTCGGACGCCGAGCTGTTCCTGCGTCTTCCTCCGGGGCCG
10261 ---------+---------+---------+---------+---------+---------+ 10320
       H  L  D  E  L  C  S  D  A  E  L  F  L  L  F  S  S  G  A  G
      GGGTGTGGGAAGCCCCCGCCAGGGCGCCTACCGCCGGGCAACGCGGTTCCTGGACCCCT
10321 ---------+---------+---------+---------+---------+---------+ 10380
       V  W  G  S  A  R  Q  G  A  Y  A  A  A  G  N  A  F  L  D  A  F
```

FIG.2-27

FIG. 2-28

```
10381 TCGCCCGCACCGCCCGGGGCCGCCTCCCCGCCACGTCGGTGGGCGTCGTGGG
      ------+---------+---------+---------+---------+ 10440 -
       A   R   H   R   R   G   R   G   L   P   A   T   S   V   A   W   G   L   W   A

10441 CGGGGCGGCCATGACCGGCGACGAGGACGCCGTGTCGTTCCTGCGCGAGCGCGGTGTGC
      ------+---------+---------+---------+---------+ 10500 -
       A   G   G   M   T   G   D   E   E   A   V   S   F   L   R   E   R   G   V   R

10501 GGGCGATGCCCGTACCGGCGCGGCCCTCGCCGCCCTGGACAGGGTGCTGGCCTCCGGGGAGA
      ------+---------+---------+---------+---------+ 10560 -
       A   M   P   V   P   R   A   L   A   A   L   D   R   V   L   A   S   G   E   T

10561 CGGCGGTGGTCGTGACGGACGTGGACTGGCCGGCCTTCGCCGAGTCCTACACCGCCGCCC
      ------+---------+---------+---------+---------+ 10620 -
       A   V   V   V   T   D   V   D   W   P   A   F   A   E   S   Y   T   A   A   R

10621 GGCCCCGGCCGTTGCTCGACCGCATCGTCACGACCGCGCCGAGCGAGCGGGCCGGAGAAC
      ------+---------+---------+---------+---------+ 10680 -
       P   R   P   L   L   D   R   I   V   T   T   A   P   S   E   R   A   G   E   P

10681 CGGAGACGGAGAGCCTGCGCGACCGGCTGGCCGGGCTGCCGCGTGCCGAGCGGACGGCGG
      ------+---------+---------+---------+---------+ 10740 -
       E   T   E   S   L   R   D   R   L   A   G   L   P   R   A   E   R   T   A   E
```

FIG. 2-29

```
10741 AGCTTGGTGCGCCTGCTGGTCCGCCACCAGCACCGGACCGTGCTGGCCGACGACCCGAAGG 10800
      ---------+---------+---------+---------+---------+---------+
        L   V   R   L   V   R   T   S   T   A   T   V   L   G   H   D   D   P   K   A   -

10801 CGGTGCCGCGCCGACCACGCCGTTCAAGGAGCTCGGGTTCGACTCGCTGGCCGCCGTCCGGC 10860
      ---------+---------+---------+---------+---------+---------+
        V   R   A   T   T   P   F   K   E   L   G   F   D   S   L   A   A   V   R   L   -

10861 TGCGCCAACCTGCTCAACGGCGGCCACCGGCTCCGCCTGCCGACGCGTGGTCTTCGACC 10920
      ---------+---------+---------+---------+---------+---------+
        R   N   L   L   N   A   A   T   G   L   R   L   P   S   T   L   V   F   D   H   -

10921 ACCCGAACGGCCTCCCGCGGTCGCCGGTTTCCTCGACGCCGAGCTCGGCACCGAGGTCCGGG 10980
      ---------+---------+---------+---------+---------+---------+
        P   N   A   S   A   V   A   G   F   L   D   A   E   L   G   T   E   V   R   G   -

10981 GGGAGGCGCCGTCGCGGCCCTCGGCCCTGGACGCGCTGGAAGGCGCCCTGCCCGAGTGC 11040
      ---------+---------+---------+---------+---------+---------+
        E   A   P   S   A   L   A   G   L   D   A   L   E   G   A   L   P   E   V   P   -

11041 CCGCAACCGAGCGGGGAAGAGCTCGTGTACAGCGCGTTGGAACGGATGCTCGCGCGCTACGCC 11100
      ---------+---------+---------+---------+---------+---------+
        A   T   E   R   E   E   L   V   Q   R   L   E   R   M   L   A   A   L   R   P   -
```

FIG.2-30

```
11101 CGGTCGCCCAGGCCGCGACGCCTTCCGGGACCGGCGCCAACCCGTCCGGCGACGACCTGG
      ---------+---------+---------+---------+---------+---------+ 11160
       V  A  Q  A  A  D  A  S  G  T  G  A  N  P  S  G  D  D  L  G  -

11161 GCGAGGCGGGCGTGGACGAACTGCTCGAAGCACTCGGCCCGGGAGCTCGACGGCGATTGA
      ---------+---------+---------+---------+---------+--------- 11219
       E  A  G  V  D  E  L  L  E  A  L  G  R  E  L  D  G  D  *

12643 CCGCCGATTGGAGAAAAGGTGACTGACAGCGAGAAGGTGGCGGAGTACCTCCGTCGGGCG
      ---------+---------+---------+---------+---------+---------+ 12702
             V  T  D  S  E  K  V  A  E  Y  L  R  R  A  -

12703 ACGCTCGACCTGCTGCGTGCCGCCGGATCCGGAGCTGGAATCCGACCCGATCGCC
      ---------+---------+---------+---------+---------+---------+ 12762
       T  L  D  L  R  A  A  R  Q  R  I  R  E  L  E  S  D  P  I  A  -

12763 ATCGTCAGCATGGCCTGCCGCCTGCCGGGCGGGGTGAACACCCCGCAGCGGCTGTGGGAG
      ---------+---------+---------+---------+---------+---------+ 12822
       I  V  S  M  A  C  R  L  P  G  G  V  N  T  P  Q  R  L  W  E  -

      CTGCTCGGCGAGGGCGGTGAGACGCGTGTCGGCGTTCCCCACCAGGCGGGCTGGGACCTG
```

FIG.2-31

```
12823 --------+---------+---------+---------+---------+---------+-- 12882
        L    L   R   E   G   G   E   T   L   S   G   F   P   T   D   R   G   W   D   L
      GCGGGCTGCCACCACCCCGGACAACCCGGTACCAGCTACGTCGACAAGGGCGGG
12883 --------+---------+---------+---------+---------+---------+-- 12942
        A    R   L   H   H   P   D   P   D   N   P   G   T   S   Y   V   D   K   G   G
      TTCCTGACGACGGCGGGCTTCGACGCGGAGTTCTTCGGCGTCTCGCCGCGCGAGGGC
12943 --------+---------+---------+---------+---------+---------+-- 13002
        F    L   D   D   A   A   G   F   D   A   E   F   F   G   V   S   P   R   E   A
      GCGGCCATGGACCCGCAGCAGCGGCTGCTGCTGGAGACGAGCTGGTGGTGGAGAAC
13003 --------+---------+---------+---------+---------+---------+-- 13062
        A    A   M   D   P   Q   Q   R   L   L   L   E   T   S   W   E   L   V   E   N
      GCCCGCATCGACCCGCACTCGCTGCGCGGGTACCGCCGCGTCTTCCTCGGAGTGGCG
13063 --------+---------+---------+---------+---------+---------+-- 13122
        A    G   I   D   P   H   S   L   R   G   T   A   T   G   V   F   L   G   V   A
      AAGTTCGGCTACGGCGAGGACACCGCCGCGGCGGAGGTCGAGGGCTACTCGGTCACC
13123 --------+---------+---------+---------+---------+---------+-- 13182
        K    F   G   Y   G   E   D   T   A   A   A   E   D   V   E   G   Y   S   V   T
```

FIG.2-32

```
13183 GGTGTGGCGCCCGCGGTCGCCTCCGCCCATCCTACACCATGGGCCTGGAGGGGCCG 13242
      ------+---------+---------+---------+---------+---------+-
       G  V  A  P  A  V  A  S  G  R  I  S  Y  T  M  G  L  E  G  P

13243 TCGATCAGCGTCGACACCGCGTGCTCGTCGCTGGTGGCGCTGCACCTGGCGGTGAG 13302
      ------+---------+---------+---------+---------+---------+-
       S  I  S  V  D  T  A  C  S  S  L  V  A  L  H  L  A  V  E

13303 TCGCTGCGCAAGGGCGAGTCGTCGATGGCGGTCGTCGGCGCGGTGATGGCGACC 13362
      ------+---------+---------+---------+---------+---------+-
       S  L  R  K  G  E  S  S  M  A  V  V  G  G  A  A  V  M  A  T

13363 CCGGGGGTGTTCGTCGACTTCAGCCGGCAGCGCGCCCTCGCCGCCGACGGCGGTCGAAG 13422
      ------+---------+---------+---------+---------+---------+-
       P  G  V  F  V  D  F  S  R  Q  R  A  L  A  A  D  G  R  S  K

13423 GCGTTCGGTGCCGGCGGGTTCGGCTTCTCCGAAGGCGTCACCCTGGTCCTGCTC 13482
      ------+---------+---------+---------+---------+---------+-
       A  F  G  A  G  A  D  G  F  S  E  G  V  T  L  V  L  L

13483 GAGCGGCTGTCGGAGGCGCGGAAACGGGCACGAGGTGCTGGCGGTGGTTCGCGGCTCG 13542
      ------+---------+---------+---------+---------+---------+-
       E  R  L  S  E  A  R  R  N  G  H  E  V  L  A  V  V  R  G  S
```

FIG.2-33

```
13543 GCGCTCAACCAGGACGGGGCCAGCAACGGGCTTTCCGCGCCGAGCGGGCCCCGCGCAGCGC 13602
      --------+---------+---------+---------+---------+---------+
       A  L  N  Q  D  G  A  S  N  G  L  S  A  P  S  G  P  A  Q  R

13603 AGGGTCATCCGGCAGGCCCTCGAGAGCTGCGGTCTGGAGCCCGGCGACGTCGACGGCGGTG 13662
      --------+---------+---------+---------+---------+---------+
       R  V  I  R  Q  A  L  E  S  C  G  L  E  P  G  D  V  D  A  V

13663 GAGGCGCCACGGCCACCGTACGGCGCTCGGCGCCGATCGAGGCGGCGAACGCGCTGCTGGAC 13722
      --------+---------+---------+---------+---------+---------+
       E  A  H  G  T  G  T  A  L  G  D  P  I  E  A  N  A  L  L  D

13723 ACCTACGGCGCGGACCCGAGCGCCGACGTCTCGGCGTGGGCTCGGTGAAGTCCAAC 13782
      --------+---------+---------+---------+---------+---------+
       T  Y  G  R  D  A  D  R  P  L  W  L  G  S  V  K  S  N

13783 ATCGGCCACACCCAGGCGGCGGCGGCGTCACCGGCCGCCTGCTGAAGGTGGTCCTGGCGCTG 13842
      --------+---------+---------+---------+---------+---------+
       I  G  H  T  Q  A  A  A  G  V  T  G  L  L  K  V  V  L  A  L

13843 CGCAACGGGGAACTGCCCGCGACCCTGCACGTCGAGGAGCCCACGCCGCACGTCGACTG 13902
      --------+---------+---------+---------+---------+---------+
       R  N  G  E  L  P  A  T  L  H  V  E  E  P  T  P  H  V  D  W
```

FIG. 2-34

```
13903 TCGTCCGGCGGGCGTGGCGCTGCTGGGCGGCAACCAGCCGTGGCGGCGGGGCGAGCGGACT 13962
      ----+----+----+----+----+----+----+----+----+----+----+----+-
13963  S   S   G   G   V   A   L   L   A   G   N   Q   P   W   R   R   G   E   R   T

       CGGCGCGCCCGTGTTTCCGGTTCGGGATCAGCGGGACGAATGCGCACGTGATCGTCGAG 14022
      ----+----+----+----+----+----+----+----+----+----+----+---
14023  R   R   A   R   V   S   A   F   G   I   S   G   T   N   A   H   V   I   V   E

       GAAGCTCCTGAGCGCGAGCACCGGAGACCACCGCGCACGACGGCCGACCGGTTCCGCTG 14082
      ----+----+----+----+----+----+----+----+----+----+----+---
14083  E   A   P   E   R   E   H   R   E   T   T   A   H   D   G   R   P   V   P   L

       GTGGTGTCCGCGCGCACGACGGCGGCGTTGCGGGCCCAGCCGCGCCAGATCGCCGAGCTG 14142
      ----+----+----+----+----+----+----+----+----+----+----+----+
14143  V   V   S   A   R   T   T   A   A   L   R   A   Q   A   A   Q   I   A   E   L

       CTCGAACGCCCGGACGCCGACCTCGCCGGGGTCGGGCTGGGCCTGGCCACGACCCGGCGCC 14202
      ----+----+----+----+----+----+----+----+----+----+----+----+-
14203  L   E   R   P   D   A   D   L   A   G   V   G   L   A   T   T   R   A

       CGCCAGCGACCACCCGCCCCGGTGGTGGCATCGACCGGAGAAGCGGTGCGCGGACTG 14262
      ----+----+----+----+----+----+----+----+----+----+----+--
```

FIG.2-35

```
      R   E   H   R   A   A   V   V   A   S   T   R   E   E   A   V   R   G   L                14322
      CGGGAGATCGCCCGGTGCCCGACGGCCGTGGTCGAGGGCGTCACCGAGGTG
14263 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+--

      R   E   I   A   A   G   A   A   T   A   D   A   V   V   E   G   V   T   E   V            14382
      GACGGGCCAACGTCGTCTTCCTGTTCCCGGGGCAGGGTTCGCAATGGCCGGCATGGGT
14323 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+--

      D   G   R   N   V   V   F   L   F   P   P   G   Q   G   S   Q   W   A   G   M   G        14442
      GCCGAGCTGCTGTCGTCTCGGCCGGTGTTCGCCGGGAAGATCCGGGCCTGCGACGAGTCG
14383 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+--

      A   E   L   L   S   S   P   V   F   A   G   K   I   R   A   C   D   E   S                14502
      ATGGCCCCGATGCAGGACTGGAAGGTCTCCGACGTGCTGCGTCAGGCGCCCGGGGGCCCG
14443 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+--

      M   A   P   M   Q   D   W   K   V   S   D   V   L   R   Q   A   P   G   A   P            14562
      GGCCTGGACCGGGTCGACCTGGTGCAGCCGGTGTTGTTCGCCGGTGATGTGTCGCTGGCG
14503 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+--

      G   L   D   R   V   D   V   V   Q   P   V   L   F   A   V   M   V   S   L   A            14622
      GAGCTGTGGCGCCTCGTACGGCGTGGAGCCCGGCGGTCGTGGGCACTCGCAGGCCGAG
14563 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+--
```

FIG.2-36

```
E   E L W R S Y G V E P A A V V G H S Q G E
14623 ATCGCGCGCACGTCCGCGGGCGCTCACGTTGGAGGACGCGGGAAGCTCGTCGTG
      -----+---------+---------+---------+---------+---------+--  14682

I   I A A A H V A G A L T L E D A A K L V V
14683 GGCCGCAGCCCTGATGCCGGTCGCTCCTCCGGGGAGGGCGGCATGGCCGCCGTCGCCCTG
      -----+---------+---------+---------+---------+---------+--  14742

G   G R S R L M R S L S G E G G M A A V A L
14743 GGCGAGGCCGGCGGTGCCGGTCGCGGCCTGCGGCCGTGGCAGGACCGGCTCTCGGTGGCCGCG
      -----+---------+---------+---------+---------+---------+--  14802

G   G E A A V R E R L R P W Q D R L S V A A
14803 GTCAACGGTCCCCCGGTCTGGTGTCTCCGGCGAGCCCGGCGCTGGGGGCGTTTTCC
      -----+---------+---------+---------+---------+---------+--  14862

V   V N G P R S V V V S G E P G A L R A F S
14863 GAGGACTGCGGCGGCCCAGGGCCATCGCGTCCGGACATCGACGTGGACTACGGCCTCGCAC
      -----+---------+---------+---------+---------+---------+--  14922

E   E D C A A E G I R V R D I D V D Y A S H
```

FIG.2-37

```
14923 TCGCCCAGATCGAGCGGGTCCGGGAGGAACTCCTCGAAACGACCGGCCATCGCCCCG 14982
      ---+---------+---------+---------+---------+---------+--- -
14983  S  P  Q  I  E  R  V  R  E  E  L  L  E  T  T  G  D  I  A  P

      CGCCCCGGCGGGGTGACGTTCCACTCCACTGTGGAGTCGCGGTCTATGGACGGCACCGAG 15042
      ---+---------+---------+---------+---------+---------+--- -
15043  R  P  A  R  V  T  F  H  S  T  V  E  S  R  S  M  D  G  T  E

      CTGGATGCCCGTACTGGTACCGCAACCTGCGCGAGACGGTGCGCTTCGCCGACGCCGTG 15102
      ---+---------+---------+---------+---------+---------+--- -
15103  L  D  A  R  Y  W  Y  R  N  L  R  E  T  V  R  F  A  D  A  V

      ACGCGCTGGCCGAGTCGGGATACGACGCCGTTCATCGAGGTCAGCCCGCATCCGGTCGTG 15162
      ---+---------+---------+---------+---------+---------+--- -
15163  T  R  L  A  E  S  G  Y  D  A  F  I  E  V  S  P  H  P  V  V

      GTCCAGGCCGTCGAGGAGGCTGCGAAGAGGCTGACGGTGCCGAAGACGCGGGTCGTAGTC 15222
      ---+---------+---------+---------+---------+---------+--- -
15223  V  Q  A  V  E  E  A  A  E  D  G  A  E  D  A  V  V  V

      GGCTCGCTCCACCGGCGACGGCGGTGACCTCTCGGCCTTCCTGCGGTCGATGGCCACCGCG 15282
      ---+---------+---------+---------+---------+---------+--- -
       G  S  L  H  R  D  G  G  D  L  S  A  F  L  R  S  M  A  T  A
```

FIG.2-38

```
15283  CACGTGTCCGGTGTGGACATCAGGTGGGACGTCGCTCTGCCCGGCGCCGGCCCTTCGCG  15342
       ----+----+----+----+----+----+----+----+----+----+----+----+
        H   V   S   G   V   D   I   R   W   D   V   A   L   P   G   A   A   P   F   A

15343  CTGCCGACGTATCCGTTCCAGCGCCAAGCCGCTACTGGCTCCAGCCCGCCGCCACCCGCCGCC  15402
       ----+----+----+----+----+----+----+----+----+----+----+----+
        L   P   T   Y   P   F   Q   R   K   R   Y   W   L   Q   P   A   A   P   A   A

15403  GCCTCCGACGAGCTGGCCTACCGCGTTTCCTGGACTCCGATCGAAAAGCCGAGTCGGGA  15462
       ----+----+----+----+----+----+----+----+----+----+----+----+
        A   S   D   E   L   A   Y   R   V   S   W   T   P   I   E   K   P   E   S   G

15463  AACCTGGACGGCGACTGGTTGGTTGTCACACCCCTCATCAGTCCGGAGTGGACGGAAATG  15522
       ----+----+----+----+----+----+----+----+----+----+----+----+
        N   L   D   G   D   W   L   V   V   T   P   L   I   S   P   E   W   T   E   M

15523  CTGTGCGAGGCCATCAACGCCAACGGTGCCAGGGCGTTGCGCTGCGAGGTGGACACGTCC  15582
       ----+----+----+----+----+----+----+----+----+----+----+----+
        L   C   E   A   I   N   A   N   G   G   R   A   L   R   C   E   V   D   T   S

15583  GCTTCGCGCACTGAGATGGCCCAGGCCGTCGCACAGGCCGGAACGGGATTCCGGGGCGTG  15642
       ----+----+----+----+----+----+----+----+----+----+----+----+
```

FIG.2-39

```
        A   S   R   T   E   M   A   Q   A   V   A   Q   A   G   T   G   F   R   G   V
15643   CTCTCGTTGCTGTCGTCGGACGAATCCGCCTGCCGTCCGGGGGTTCTTGCCCGTGCCGTC   15702
        ---------+---------+---------+---------+---------+---------+

        L   S   L   L   S   S   D   E   S   A   C   R   P   G   V   P   A   G   A   V
15703   GGCCTGCTCACCCTGGTCCAGGCGCTGGGCGATGCCGGGGTCGACGCACCGGTGTGGTGC   15762
        ---------+---------+---------+---------+---------+---------+

        G   L   L   T   L   V   Q   A   L   G   D   A   G   V   D   A   P   V   W   C
15763   CTGACCCAGGGTGCGGTCCGCACTCCGGCCGACGACCTCGCCCGGCCTGCCGCAGACC   15822
        ---------+---------+---------+---------+---------+---------+

        L   T   Q   G   A   V   R   T   P   A   D   D   D   L   A   R   P   A   Q   T
15823   ACCGCGCACGGCTTCGCGCAGGTCGCGGGCTGGAGCTGCCGGGCCGTGGGGCCGGTGTG   15882
        ---------+---------+---------+---------+---------+---------+

        T   A   H   G   F   A   Q   V   A   G   L   E   L   P   G   R   W   G   G   V
15883   GTCGACCTGCCCGAATCGGTCGACGACGCGGCGGCTGCCGTCTCGTGGCAGTCCTGCGC   15942
        ---------+---------+---------+---------+---------+---------+

        V   D   L   P   E   S   V   D   D   A   A   L   R   L   L   V   A   V   L   R
15943   GGCGGCGGCCGTGCCGAGGACCACCTCGCGGTCCGGGAACGGCCGCCTCCACGGCCGTCGC   16002
        ---------+---------+---------+---------+---------+---------+
```

FIG.2-40

```
              G  G  G  R  A  E  D  H  L  A  V  R  D  G  R  L  H  G  R  R
                                                                         16062
15003  GTCGTCCGCGCAAGCTGCCAGTCCGGCTCCGGGAGCTGACCCCGCACGGGACCGTG
       -----+----+----+----+----+----+----+----+----+----+----+
       V  V  R  A  S  L  P  Q  S  G  S  R  S  W  T  P  H  G  T  V
                                                                         16122
16063  CTGGTCACCGGCGCGGGCGAGCCCCGTCGGGCGACCAACTGGTGCCGGTGGCTCGCCGACCGG
       -----+----+----+----+----+----+----+----+----+----+----+
       L  V  T  G  A  A  S  P  V  G  D  Q  L  V  R  W  L  A  D  R
                                                                         16182
16123  GGAGCCGAGCCGCTGGTGCTGGCCGGAGCCTGTCCGGGCGACGACCTGCTGGCCGCGGTC
       -----+----+----+----+----+----+----+----+----+----+----+
       G  A  E  R  L  V  L  A  G  A  C  P  G  D  D  L  L  A  A  V
                                                                         16242
16183  GAGGAGGCGGGCGCATCGGCCGTCGTGTGCGCCCAGGACGCGGCGGCGCTGCGCGAGGCG
       -----+----+----+----+----+----+----+----+----+----+----+
       E  E  A  G  A  S  A  V  V  C  A  Q  D  A  A  A  L  R  E  A
                                                                         16302
16243  CTCGGGCGACGAGCCGGTGACCGGCTCGTGCACCGCCCGAGAACCTGACGAACTTCGGCCAGC
       -----+----+----+----+----+----+----+----+----+----+----+
       L  G  D  E  P  V  T  A  L  V  H  A  G  T  L  T  N  F  G  S
       ATCAGGGAAGTCGCACCGGAGGAGTTCGCCGAGACGATCGCGGCCAAGACCGCGTTGCTC
```

FIG.2-41

```
16303 ------+-------+-------+-------+-------+-------+ 16362
      I  S  E  V  A  P  E  E  F  A  E  T  I  A  A  K  T  A  L  L

      GCCGTGCTGGACGAAGTCCTCGGCCGTGGGCCGTGCGAGCGGGAGGTCTACTGCTCGTCG
16363 ------+-------+-------+-------+-------+-------+ 16422
      A  V  L  D  E  V  L  G  D  R  A  V  E  R  E  V  Y  C  S  S

      GTCGCCGGATCTGGGGCGGCGCCGGGATGGCCGCCTACGGCGGCAGGCAGCGCCTACCTC
16423 ------+-------+-------+-------+-------+-------+ 16482
      V  A  G  I  W  G  G  A  G  M  A  A  Y  A  A  G  S  A  Y  L

      GAGCGGCTGGCCGAGCACCACCGCGGCGGGCCCCTCGTGCACCTCGGTCGCCTGGACG
16483 ------+-------+-------+-------+-------+-------+ 16542
      D  A  L  A  E  H  H  R  A  R  G  R  S  C  T  S  V  A  W  T

      CCGTGGGCGCTGCCCGGCGGGGGCGGTGGACGACGGCTACCTGCCGGGAACGCGGACTGCGC
16543 ------+-------+-------+-------+-------+-------+ 16602
      P  W  A  L  P  G  G  A  V  D  D  G  Y  L  R  E  R  G  L  R

      AGCCTCTCCGCCGACAGGGCGATGCCACCTGGGAGCGGCGTGCTGGCCGCCGGGCCGGTG
16603 ------+-------+-------+-------+-------+-------+ 16662
      S  L  S  A  D  R  A  M  R  T  W  E  R  V  L  A  A  G  P  V
```

```
16663 TCGGTCGCGGTGGCGACGTGGACTGCCCGTGCTCAGCGAAGGCTTCCGCGCCACCCGG 16722
      ------+---------+---------+---------+---------+---------+  -
       S   V   A   V   A   D   V   D   W   P   V   L   S   E   G   F   A   A   T   R

16723 CCGACCGGCTCGTTCGCCGAACTCGCCGGCCGCGGGACAGGCGGAGGCCGAGCCGGAC 16782
      ------+---------+---------+---------+---------+---------+  -
       P   T   A   L   F   A   E   L   A   G   R   G   G   Q   A   E   E   P   D

16783 AGCGGACCGACCGGCGAGCCGGCACAACGGCTCCGCGGGGCTTTCCCCGGACGAGCAGCAG 16842
      ------+---------+---------+---------+---------+---------+  -
       S   G   P   T   G   E   P   A   Q   R   L   A   G   L   S   P   D   E   Q   Q

16843 GAAAACCTGCTCGAACTCGTCGCGGAACGCCGGTTGCCGAGGTGCTTGGCCACGAGTCCGCC 16902
      ------+---------+---------+---------+---------+---------+  -
       E   N   L   L   E   L   V   A   N   A   V   A   E   V   L   G   H   E   S   A

16903 GCCGAGATCAACGTGCGCCGCGGTTCAGCGAGCTCGGACTCGACTCGCTCAACCGGATG 16962
      ------+---------+---------+---------+---------+---------+  -
       A   E   I   N   V   R   R   A   F   S   E   L   G   L   D   S   L   N   A   M

16963 GCCCTGCGCAAGCGCCTGTCGGCGAGCACCGGCCTGCGCCCGGTCGCGTGGTGTTC 17022
      ------+---------+---------+---------+---------+---------+  -
       A   L   R   K   R   L   S   A   S   T   G   L   R   L   P   A   S   L   V   F
```

FIG.2-42

FIG.2-43

```
17023                                                              17082
GACCACCCCACCGTCGCTGGGCGAGCACCTGCGCGCCCGGCTCGTCGGTGACGCC
-----+---------+---------+---------+---------+---------+---
 D   H   P   T   V   T   A   L   A   Q   H   L   R   A   R   L   V   G   D   A


17083                                                              17142
GACCAGGCCGCGGGTGCGCGTTCGTCGGCGGCCGACGAGTCCGAGCCCATCGCCATCGTC
-----+---------+---------+---------+---------+---------+---
 D   Q   A   A   V   R   V   V   G   A   A   D   E   S   E   P   I   A   I   V


17143                                                              17202
GGCATCGGCTGCCGTTTCCCCGGCGGGCATCGGCTCCGCCCGAGCAGTTGTGGCGGGTGCTG
-----+---------+---------+---------+---------+---------+---
 G   I   G   C   R   F   P   G   G   I   G   S   P   E   Q   L   W   R   V   L


17203                                                              17262
GCCGAGGGCGGGAACCTCACCACCGGCTTCCCGGCCGACCGGGGCTGGGACATCGGCGCGG
-----+---------+---------+---------+---------+---------+---
 A   E   G   A   N   L   T   T   G   F   P   A   D   R   G   W   D   I   G   R


17263                                                              17322
CTCTACCACCCGGACCCGGACAACCCGGCCACCAGCTACGTGGACAAGGGCGGGTTCCTC
-----+---------+---------+---------+---------+---------+---
 L   Y   H   P   D   P   D   N   P   G   T   S   Y   V   D   K   G   G   F   L


17323                                                              17382
ACCGACGCGGCGGATTTCGACCCGGGCTTCTTCGGCCATCACCGCCCCGGAAGCCGCTGGCG
-----+---------+---------+---------+---------+---------+---
 T   D   A   A   D   F   D   P   G   F   F   G   I   T   P   R   E   A   L   A
```

FIG. 2-44

```
17383 ATGGACCCGCAGCAGCCCTCATGCTGGAGACGCGGGCGTGGAGGCAGTGGAACGCCGGGGC 17442
      ------+---------+---------+---------+---------+---------+-
       M  D  P  Q  Q  R  L  M  L  E  T  A  W  E  A  V  E  R  A  G

17443 ATCGACCCCGACGCCCTGCGAGGCACCGACACCGGCGTCTTCGTCGGCATGAACGGCCAG 17502
      ------+---------+---------+---------+---------+---------+-
       I  D  P  D  A  L  R  G  T  D  T  G  V  F  V  G  M  N  G  Q

17503 TCCTACATGCAGCTGCTGGCCGGTGAGGCCGAACGGGTCGACGGCTACCAGGGCCTCGGA 17562
      ------+---------+---------+---------+---------+---------+-
       S  Y  M  Q  L  L  A  G  E  A  E  R  V  D  G  Y  Q  G  L  G

17563 AACTCCGCGAGCGTGCTCTCCGGGCGCATCGCCTACACCTTCGGCTGGGAGGGCCCGGCG 17622
      ------+---------+---------+---------+---------+---------+-
       N  S  A  S  V  L  S  G  R  I  A  Y  T  F  G  W  E  G  P  A

17623 CTGACGGTGGACACCGCGTGCTCGTCCTCGCTGGTCGGCATCCACCTCGCCGATGCAGGCG 17682
      ------+---------+---------+---------+---------+---------+-
       L  T  V  D  T  A  C  S  S  S  L  V  G  I  H  L  A  M  Q  A

17683 CTGCGGCGCGGTGAGTCGTCTCCCTGGCCGGCTGCGGGCGTCACCGTCATGTCCGACCCG 17742
      ------+---------+---------+---------+---------+---------+-
       L  R  R  R  G  W  E  G  P  A  M  A  Q  A
```

FIG.2-45

```
17743                                                                    17802
L   R   R   G   E   C   S   L   A   L   A   G   G   V   T   V   M   S   D   P
TACACCTTCGTCGACTTCAGCAGCGCGGGCTCGCCTCCGACGGTCGCTGCAAGGCG
--+-------+-------+-------+-------+-------+-------+-------+--

17803                                                                    17862
Y   T   F   V   D   F   S   T   Q   R   G   L   A   S   D   G   R   C   K   A
TTCTCCGCGCGGCCGACGGCTTCGCGCTGTCGGAAGGCGTCGCCGCGCTGGTGCTGGAG
--+-------+-------+-------+-------+-------+-------+-------+--

17863                                                                    17922
F   S   A   R   A   D   G   F   A   L   S   E   G   V   A   A   L   V   L   E
CCGCTTTCCCGGGCGCGCCAACGGCGCACCAGGTGCTGGCCGTGCTGCGGGCAGCGCCG
--+-------+-------+-------+-------+-------+-------+-------+--

17923                                                                    17982
P   L   S   R   A   R   A   N   G   H   Q   V   L   A   V   L   R   G   S   A
GTCAACCAGGACGGTGCCAGCAACGGTCTCGCCGCTCCAACGGCCCGTCGCAGGAGCGG
--+-------+-------+-------+-------+-------+-------+-------+--

17983                                                                    18042
V   N   Q   D   G   A   S   N   G   L   A   A   P   N   G   P   S   Q   E   R
GTGATCCGGCAGGCGCTCGCCCGCTTCGGGCCGTGCCGCGGGACGTCGACGTCGTGGAG
--+-------+-------+-------+-------+-------+-------+-------+--

V   I   R   Q   A   L   A   A   S   G   V   P   A   A   A   D   V   D   V   V   E
```

FIG.2-46

```
18043 GCGCACGGGACGGGCACCGAGTCGGCGACCCGATCGGAGGCCGGCGCTCATCGCGACC 18102
      ------+---------+---------+---------+---------+---------+   -
      A   H  G  T  G  T  E  L  G  D  P  I  E  A  G  A  L  I  A  T

18103 TACGGCCAGGACCGGCGACCGGCCGCTGCGGCTCGGCTCGGTGAAGACCAACATCGGCCAC 18162
      ------+---------+---------+---------+---------+---------+   -
      Y   G  Q  D  R  P  L  R  L  G  S  V  K  T  N  I  G  H

18163 ACCCAGGCCGCGGCGGGCCGCGGGCCGTGATCAAGGTCGTGCTGGCGATGCGGCACGGG 18222
      ------+---------+---------+---------+---------+---------+   -
      T   Q  A  A  A  G  A  A  G  V  I  K  V  V  L  A  M  R  H  G

18223 ATGCTGCCCGGTCGTTGCACGCCGACGAGCTGTCCCCGCACATCGACTGGGAGTCGGGG 18282
      ------+---------+---------+---------+---------+---------+   -
      M   L  P  R  S  L  H  A  D  E  L  S  P  H  I  D  W  E  S  G

18283 GCCGTGGAGGTGCTGCGCGAGGAGGTGCCGTGGCCGGGGTGAGCGCCCCGGCCGGGGCG 18342
      ------+---------+---------+---------+---------+---------+   -
      A   V  E  V  L  R  E  E  V  P  W  P  A  G  E  R  P  R  R  A

18343 GGGGTGTCGTCCTTCGGCGTCAGCGGAACCAACGCCACGTGATCGTCGAAGAGGCCACCA 18402
      ------+---------+---------+---------+---------+---------+   -
      G   V  S  S  F  G  V  S  G  T  N  A  H  V  I  V  E  E  A  P
```

FIG. 2-47

18403 GCAGAGCAGGAGGCCGCCCCGCACCGAGCGCGGTCCGGTTCGTGCTGTCCGGCCGC 18462
       -----+----+----+----+----+----+----+----+----+----+----+----+-- -
        A   E   Q   E   A   A   R   T   E   R   G   P   L   P   F   V   L   S   G   R

18463 AGCGAAGCCGTGGTCGGCGCCCAGGCCCGCGCTCGCGAGCACCTGCGCGACACCCCG 18522
       -----+----+----+----+----+----+----+----+----+----+----+----+-- -
        S   E   A   V   V   A   A   Q   A   R   A   L   A   E   H   L   R   D   T   P

18523 GAGCTCGGCCTGACCGACGCGGGTGGACGCTCCGACCGGCCAGGGCGGTTCGACGTG 18582
       -----+----+----+----+----+----+----+----+----+----+----+----+-- -
        E   L   G   L   T   D   A   A   W   T   L   A   T   G   R   A   R   F   D   V

18583 CGAGCCCGCCGTGCTCGGCGACACCGGCCGCGGGTGTGCCGGAGCTGGACGCGCTGGCC 18642
       -----+----+----+----+----+----+----+----+----+----+----+----+-- -
        R   A   A   V   L   G   D   D   R   A   G   V   C   A   E   L   D   A   L   A

18643 GAGGGCCGCCCCGTCGGCGACCGCGTGCCGGCGGTGACCTCCGGCGGCAAGCCGGTC 18702
       -----+----+----+----+----+----+----+----+----+----+----+----+-- -
        E   G   R   P   S   A   D   A   V   A   P   V   T   S   A   P   R   K   P   V

18703 CTGGTCTTCCCCGGCCAGGGCGCCAGTGGGTCGGCATGGCACGCGATCTGCTGGAATCC 18762
       -----+----+----+----+----+----+----+----+----+----+----+----+-- -
        L   V   F   P   G   Q   G   A   Q   W   V   G   M   A   R   D   L   L   E   S

FIG.2-48

```
18763                                                                    18822
      TCCGAGTGTTCGCCGAGTCGATGAGCCGGTGCCGGAGGCGCTCTCCGCCACACCGAC
      --------+---------+---------+---------+---------+---------+--
        S   E   V   F   A   E   S   M   S   R   C   A   E   A   L   S   P   H   T   D
18823                                                                    18882
      TGGAAGTTGCTCGACGTCGTCCGCGGCGACGGCGGTCCCGACCCGCACGAGCGCGTCGAC
      --------+---------+---------+---------+---------+---------+--

18883                                                                    18942
      GTGCTCCAGCCCGTGCTGTTCTCTCGATCATGGTCTCGCTCGGCCGGAGCTGTGGCGGCGGCCAC
      --------+---------+---------+---------+---------+---------+--
        W   K   L   L   D   V   V   R   G   D   G   G   P   D   P   H   E   R   V   D
18883                                                                    18942
      V   L   Q   P   V   L   F   S   I   M   V   S   L   A   E   L   W   R   A   H

18943                                                                    19002
      GGCGTGACCCCGGCCCCGTCGTCGGCCACTCGCAGGGCGGAGATCGCGCCGGGCCCACGTG
      --------+---------+---------+---------+---------+---------+--
        G   V   T   P   A   A   V   V   G   H   S   Q   G   E   I   A   A   A   H   V

19003                                                                    19062
      GCCGGGCCGCTGTCGCTGGAAGCGCGCCGCGAAGGTGGTGCCCTGCGCAGCCAGGTGTTG
      --------+---------+---------+---------+---------+---------+--
        A   G   A   L   S   L   E   A   A   A   K   V   V   A   L   R   S   Q   V   L
19063                                                                    19122
      CGCGAGCTCGACGACCAGGGCCATGGTGTCGGTCGGCCGCGTCCCGCGACGAGCTGGAG
      --------+---------+---------+---------+---------+---------+--
```

FIG.2-49

```
        R   E   L   D   D   Q   G   G   M   V   S   V   G   A   S   R   D   E   L   E   -
        ACCGTCCTCGCGCGCCTGGACGGGCCGTGTCGCGGTGGCGGCGTGAACGGGCCCTGGCACC
19123   ---------+---------+---------+---------+---------+---------+   19182

        T   V   L   A   R   W   D   G   R   V   A   A   V   N   G   P   G   T   -
        AGCGTCGTTGCCGGGCCGACCGCCGGAGCTGGACGAGTTCTTCGCCGAGGCCGAGGCGCGG
19183   ---------+---------+---------+---------+---------+---------+   19242

        S   V   V   A   G   P   T   A   E   L   D   E   F   F   A   E   A   E   A   R   -
        GAGATGAAGCCGCGCCGGATCGCCGTGGCCGTACGCCTCCCACTCCCCGGAGGTGGCCGCGC
19243   ---------+---------+---------+---------+---------+---------+   19302

        E   M   K   P   R   R   I   A   V   R   Y   A   S   H   S   P   E   V   A   R   -
        ATCGAGGACCGGCTCGCGGCCGAGCTGGGCACCATCACCGCCGTGCGGGGCTCGGTGCCG
19303   ---------+---------+---------+---------+---------+---------+   19362

        I   E   D   R   L   A   A   E   L   G   T   I   T   A   V   R   G   S   V   P   -
        CTGCACTCCACGGTGACCGGCGAGGTCATCGACACCTCCGCGATGGACGCCCTCCTACTGG
19363   ---------+---------+---------+---------+---------+---------+   19422

        L   H   S   T   V   T   G   E   V   I   D   T   S   A   M   D   A · S · Y   W   -
        TACCGCAACCTGCCGACCAGTGCTCTTCGAGCAGGCGGTCTGGTCGAGCAG
19423   ---------+---------+---------+---------+---------+---------+   19482
```

FIG.2-50

```
        Y   R   N   L   R   R   P   V   L   F   E   Q   A   V   R   G   L   V   E   Q
        GGCTTCGACACCTTCCTGAGGTGAGCCCGCACCCGGTGCTGCTGATGGCGGTCGAGGAG
19483   ---------+---------+---------+---------+---------+---------+---   19542

        G   F   D   T   F   V   E   V   S   P   H   P   V   L   L   M·  A   V   E   E
        ACCGCCGAGCACGCGGGCGAAGTCACCTGCGTGCCGACGCGCCGCAGGCAGAGC
19543   ---------+---------+---------+---------+---------+---------+---   19602

        T   A   E   H   A   G   A   E   V   T   C   V   P   T   L   R   R   E   Q   S
        GGACCGCCACGAGTTCCTGCGGCTCAACCTGCTGCGGGCTCACGTGCACGGCGTCGGCGCCGAC
19603   ---------+---------+---------+---------+---------+---------+---   19662

        G   P   H   E   F   L   R   N   L   L   R   A   H   V   H   G   V   G   A   D
        CTGCGTCCGGCGGTGGCCGGGGACCGGCCGAGCTGCCCACCTACCCGTTCGAACAC
19663   ---------+---------+---------+---------+---------+---------+---   19722

        L   R   P   A   V   A   G   G   R   P   A   E   L   P   T   Y   P   F   E   H
        CAGCGCTTCTGGCCGCGGCCGCACCGGCCGAGCTCTCGGGCCGCTGGCGGTGCGCGGC
19723   ---------+---------+---------+---------+---------+---------+---   19782

        Q   R   F   W   P   R   R   P   H   R   P   A   D   V   S   A   L   G   V   R   G
        GCGGAGCACCCGCCTGCTCGCGCGCGGTGCACGTGCCGGGCCACGGCCGGTGCCGGTGTTC
```

FIG.2—51

```
19783 --------+--------+--------+--------+--------+--------+-- 19842
        A  E  H  P  L  L  L  A  A  V  D  V  P  G  H  G  G  A  V  F
      ACCGGAAGCTTTCCACCGACGAGCAGCCGTGGCTCGCCGAACACGTCGTGGGCGGCCGG
19843 --------+--------+--------+--------+--------+--------+-- 19902
        T  G  R  L  S  T  D  E  Q  P  W  L  A  E  H  V  V  G  G  R
      ACGGCTGGTGCCGGGCAGCGTCCTGGTCGATCTCGCCGCTCCGCCCGGGTGAGGACGTCGGG
19903 --------+--------+--------+--------+--------+--------+-- 19962
        T  L  V  P  G  S  V  L  V  D  L  A  L  A  A  G  E  D  V  G
      CTGCCGGTTCCTGAGGAACTGGTGTTGCAACGGCCCGCTGGTGCTCGGCCGGGGCGGGGGGCG
19963 --------+--------+--------+--------+--------+--------+-- 20022
        L  P  V  L  E  E  L  V  L  Q  R  P  L  V  L  A  G  A  G  A
      CTGCTGCCGCATGTCGTCGGCGCGCCCGAGTCGGGCGCGGGACGATCGACGTCCAC
20023 --------+--------+--------+--------+--------+--------+-- 20082
        L  L  R  M  S  V  G  A  P  D  E  S  G  R  R  T  I  D  V  H
      GCCGGCGAAGACGTGGCCGACTTCGCCGACCTCGGCCAGTGGTCCCAGCACGCCCACCGGGACG
20083 --------+--------+--------+--------+--------+--------+-- 20142
        A  A  E  D  V  A  D  L  A  D  A  Q  W  S  Q  H  A  T  G  T
```

FIG. 2-52

```
20143                                                                    20202
 .CTCGCGAGGGCGTCGCCGGGTCCGAGGGATACCGAGCAGTGGCCGCCGGAGGACGCC
 ---------+---------+---------+---------+---------+---------+--
  L  A  Q  G  V  A  A  G  P  R  D  T  E  Q  W  P  P  E  D  A
 GTCCGCATCCCGCTGACGACCACTACGACGGCCTCGCCGAGCAGGCTACGAGTACGGA
20203

20203                                                                    20262
 ---------+---------+---------+---------+---------+---------+--
  V  R  I  P  L  D  D  H  Y  D  G  L  A  E  Q  G  Y  E  Y  G
 CCGTCGTTCCAGGCCCTGCGAGCCGCGTGGCGCCAAGGACGACTCGGTCTACGCCGAGGTG
20263

20263                                                                    20322
 ---------+---------+---------+---------+---------+---------+--
  P  S  F  Q  A  L  R  A  A  W  R  K  D  D  S  V  Y  A  E  V
 TCCATCGCGGCGGACGAGGAAGTTACGCGTTCCACCCGGTGCTGCTGGACGCCGGTGGCG
20323

20323                                                                    20382
 ---------+---------+---------+---------+---------+---------+--
  S  I  A  A  D  E  E  G  Y  A  F  H  P  V  L  L  D  A  V  A
 CAGACGCTCAGCCTGGGCGCCCTCGGGCGAGCCGGCGGGGAAAGCTGCCGTTCGCCGTGG
20383

20383                                                                    20442
 ---------+---------+---------+---------+---------+---------+--
  Q  T  L  S  L  G  A  L  G  E  P  G  G  G  K  L  P  F  A  W
 AACACCGTGACCCTGCACCGCTCCGGGGCGACCTCGGTGCGGGTGCGGACCCCGGCC
20443

20443                                                                    20502
 ---------+---------+---------+---------+---------+---------+--
  N  T  V  T  L  H  A  S  G  A  T  S  V  R  V  V  A  T  P  A
```

```
20503  GGGGCGGACGCGGATGGCCCTGCGGGTCACCGACCCGGCAGGCCACCTGTCGCCACGGTC  20562
       -----+---------+---------+---------+---------+---------+
        G  A  D  A  M  A  L  R  V  T  D  P  P  A  G  H  L  L  V  A  T  V   -

20563  GACTCGCTGGTCGTCGTCCGCAGCACCGGGGAGAAGTGGGAGCAGCCGCGCGGGGGTGGC  20622
       -----+---------+---------+---------+---------+---------+
        D  S  L  V  V  R  S  T  G  E  K  W  E  Q  P  P  R  G  G   -

20623  GAGGGCGAGCTGCACGCTCTGGACTGGGGACGGCTAGCCGAGCCCGGCTCGACCGGTCGT  20682
       -----+---------+---------+---------+---------+---------+
        E  G  E  L  H  A  L  D  W  G  R  L  A  E  P  G  S  T  G  R   -

20683  GTGGTCGCGGCCGATGCCTCGGACCTCGACGCCGTCCTGCGGGTCCGGTGAACCCGAACCC  20742
       -----+---------+---------+---------+---------+---------+
        V  V  A  A  D  A  S  D  L  D  A  V  L  R  S  G  E  P  E  P   -

20743  GACGCGGTCTGGTCCGCTACGAAGGCGACGACCCCCGGCCGGGCGGCCGCCCGCCAC  20802
       -----+---------+---------+---------+---------+---------+
        D  A  V  L  V  R  Y  E  P  E  G  D  D  P  R  A  A  A  R  H   -

20803  GGGCTCCTCTGGGCCGCCGGCTCGTCGCCGCTGCTCGAACAGGAGGAGCTGCCCGGGC  20862
       -----+---------+---------+---------+---------+---------+
```

FIG.2-53

FIG.2-54

```
        G   V   L   W   A   A   A   L   V   R   R   W   L   E   Q   E   E   L   P   G
20863   GCGACGCTGGTCATCGCCGCGGCGCTGGTCCGGCGGCGGTGGCTACCGTGTCCGACGACGACAGCGTTCCC
        ---------+---------+---------+---------+---------+---------+---------+  20922

        A   T   L   V   I   A   T   S   G   A   V   T   V   S   D   D   D   S   V   P
20923   GAACCCGGCCGCCGCGGATGTGGGGCGTGATCCGCTGTGCCCAGGCCGAGTCGCCGGAC
        ---------+---------+---------+---------+---------+---------+---------+  20982

        E   P   G   A   A   A   M   W   G   V   I   R   C   A   Q   A   E   S   P   D
20983   CGGTTCGTGCTCCTCGACACCGACGCGGAACCTGGATCGCCTGCCGGTTCCGGACAAC
        ---------+---------+---------+---------+---------+---------+---------+  21042

        R   F   V   L   L   D   T   D   A   E   P   G   M   L   P   A   V   P   D   N
21043   CCGGCAGCTCGGCTTGCGCGGGCGACGACGCGTCTTCGTGCCGGCCCTCTCGCCGCTCGCACCT
        ---------+---------+---------+---------+---------+---------+---------+  21102

        P   Q   L   A   L   R   G   D   D   V   F   V   P   R   L   S   P   L   A   P
21103   TCCGGCGCTGACGCTTCCGGCAGGCACCCAACGTCTCGTGCCGGGTGACGGGGCGATCGAC
        ---------+---------+---------+---------+---------+---------+---------+  21162

        S   A   L   T   L   P   A   G   T   Q   R   L   V   P   G   D   G   A   I   D
21163   TCCGTGGCCTTCGAGCCGCACCGACGTCGAGCAGCGTCCGAGCGGGCGGCGGAGGTCCGG
        ---------+---------+---------+---------+---------+---------+---------+  21222
```

FIG.2—55

```
      S     V   A   F   E   P   A   P   D   V   E   Q   P   L   R   A   G   E   V   R
21223 GTGGACGTGCGCGCCACCGGAGTCAACTTCCGGACGTCCTCCTCGCACTCGGCATGTAT 21282
      ---------+---------+---------+---------+---------+---------+

      V     D   V   R   A   T   G   V   N   F   R   D   V   L   L   A   L   G   M   Y
21283 CCCCAGAAGGCGGACATGGGCACCGAGGCCCCGGTGTCGTCACCGGCGGTCGGACCGGAC 21342
      ---------+---------+---------+---------+---------+---------+

      P     Q   K   A   D   M   G   T   E   A   A   G   V   V   T   A   V   G   P   D
21343 GTGGACGCCTTCGCGCCGGGAGACCGGGTGCTCGGCCTGTTCCAGGGAGCCTTCGCGCCG 21402
      ---------+---------+---------+---------+---------+---------+

      V     D   A   F   A   P   G   D   R   V   L   G   L   F   Q   G   A   F   A   P
21403 ATCGCGGTCACCGATCACCGGCTCCTCGCACGAGTGCCGGACGGCTGGAGCGACGCCGAC 21462
      ---------+---------+---------+---------+---------+---------+

      I     A   V   T   D   H   R   L   L   A   R   V   P   D   G   W   S   D   A   D
21463 GCCGCGGCCGTGCCCATCGCCTACACCACCGGCCATTACGCGCTGCACGATCTCGCGGGG 21522
      ---------+---------+---------+---------+---------+---------+

      A     A   A   V   P   I   A   Y   T   T   A   H   Y   A   L   H   D   L   A   G
21523 CTGCGCGGCGGGTCAGTCGGTGCTCATCCACCGCAGCCGGCCAGGCGGTGTCGGCATGGCC 21582
      ---------+---------+---------+---------+---------+---------+
```

FIG.2-56

```
     L R A G Q S V L I H A A A G G V G M A A
     GTCGCCGCTGGCCGCCGCGAGCCGGGGGGGGGAGTGTTGGCCACCGCCCGGCCCGGCCAAGCAC
21583 ----+----+----+----+----+----+----+----+----+----+----+----+----  21642

     V A L A R R A G A E V L A T A G P A K H
     GGGACGCCTGCGGGCGCCTCGGTCTCGACGACGAGCACATCGCTTCCTCCCGGGAGACCGGT
21643 ----+----+----+----+----+----+----+----+----+----+----+----+----  21702

     G T L R A L G L D D E H I A S S R E T G
     TTCGCCCGGAAGTTCCGGGAGCGCACCGGGAGGCCCGTGGACGTGGTGCTCAACTCG
21703 ----+----+----+----+----+----+----+----+----+----+----+----+----  21762

     F A R K F R E R T G G R G V D V V L N S
     CTCACCGGGGAACTGCTCGACGAGTCCGCGGATCTGCTCGCCGAGGACGGCGTCTTCGTC
21763 ----+----+----+----+----+----+----+----+----+----+----+----+----  21822

     L T G E L L D E S A D L L A E D G V F V
     GAGATGGGCAAGACCGACCTGCGCGGGGACGCCGGGGACTTCCGGGGCCGATACGCCCCGTTC
21823 ----+----+----+----+----+----+----+----+----+----+----+----+----  21882

     E M G K T D L R D A G D F R G R Y A P F
```

FIG.2-57

```
21883                                                                           21942
D    L    G    E    A    G    D    D    R    L    G    E    I    L    R    E    V    V    G    L
GACCTCGGCGGAGGCGGGTGACGACCGGCTCGGGGAGATCCTCGCGCGAGGTCGTCGGCCTG
-----+---------+---------+---------+---------+---------+---------+-----

21943                                                                           22002
L    G    A    G    E    L    D    R    L    P    V    S    A    W    E    L    G    S    A    P
CTGGGCGCCGGGGAGCTCGACCGGCTCCCGGTATCGGCGTGGGAGCTGGGATCGCGCCCC
-----+---------+---------+---------+---------+---------+---------+-----

22003                                                                           22062
A    A    L    Q    H    M    S    R    G    R    H    V    G    K    L    V    L    T    Q    P
GCGGCCGTTGCAGCACATGAGCCGGGGCAGGCACGTCGGCAAGCTCGTGCTGACCCAGCCC
-----+---------+---------+---------+---------+---------+---------+-----

22063                                                                           22122
A    P    V    D    P    D    G    T    V    L    I    T    G    G    T    L    G    R
GCCCCGGTGGACCCCGACGGCACGGTGCTGATCACGGGTGGCACCGGCACGCTCGGACGG
-----+---------+---------+---------+---------+---------+---------+-----

22123                                                                           22182
L    L    A    R    H    L    V    T    E    H    G    V    R    H    L    L    L    V    S    R
CTGCTCGCGCGCCACCTCGTCACCGAGCACGGCGTGCGCCACCTGCTCGTGGTCAGCAGG
-----+---------+---------+---------+---------+---------+---------+-----

22183                                                                           22242
CGCGGGCGGGACGCGCCGGTTCCGACGAGCTGCGCGGCGGAGATCGAGGACTTGGGCGCG
-----+---------+---------+---------+---------+---------+---------+-----
```

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FIG. 2-58

```
          R   G   A   D   A   P   G   S   D   E   L   R   A   E   I   E   D   L   G   A
22243  TCCGCGGAGATCGCGGCTTGCCGACACCGCCCGACCGCGCTTTCGGCCGCTGCTGGAC  22302
       ---------+---------+---------+---------+---------+---------+

          S   A   E   I   A   A   C   D   T   A   D   R   D   A   L   S   A   L   L   D
22303  GGGCTGCCCCCGGCCGCTGACCGGTGTCGTGCACGCGCGGGGTGTGCTGGCCGACGGGCTG  22362
       ---------+---------+---------+---------+---------+---------+

          G   L   P   R   P   L   T   G   V   V   H   A   A   A   G   V   L   A   D   G   L
22363  GTCACCTCCATCGACGAGCGGCGGTGGAGCAGGTGCTGCGCGCCAAGGTCGACGCGGCG  22422
       ---------+---------+---------+---------+---------+---------+

          V   T   S   I   D   E   P   A   V   E   Q   V   L   R   A   K   V   D   A   A
22423  TGGAACCTGCACGAGCTGACGGGCAACACCGGTCTCTTCGTGCTGTTCTCGTCC  22482
       ---------+---------+---------+---------+---------+---------+

          W   N   L   H   E   L   T   A   N   T   G   L   S   F   F   V   L   F   S   S
22483  GCGGGCGTCGGTGCTAGCCGGGCCAGGGCGTGTACGCGGCCGGAACGAGTCGCTC  22542
       ---------+---------+---------+---------+---------+---------+

          A   A   S   V   L   A   G   P   G   Q   G   V   Y   A   A   A   N   E   S   L
22543  AACGGCCTGCTGCCCTCCGGAGGACGGCGGCCTTCCCGCGAAGGCGCTCGGATGGGGA  22602
       ---------+---------+---------+---------+---------+---------+
```

FIG.2—59

```
        N   A   L   A   A   L   R   R   T   R   G   L   P   A   K   A   L   G   W   G
        CTGTGGGCCAGGCCAGCGAGATGACCAGCGACTCGGCCGACCCATCGCCCGGACCGGG
22603   -----+----+----+----+----+----+----+----+----+----+----+----+   22662


        L   W   A   Q   A   S   E   M   T   S   G   L   G   D   R   I   A   R   T   G
        GTCGCCGGCCTGCCGACCGAGCGGGGCTCGCACTGTTCGACACGCCCTGCGCCGCGGC
22663   -----+----+----+----+----+----+----+----+----+----+----+----+   22722


        V   A   A   L   P   T   E   R   A   L   F   D   S   A   L   R   R   G
        GGTGAGGTCGTGTTCCCGTCCATCAACCGTTCCGCGCTGCGCAGGGCCGAGTTCGTG
22723   -----+----+----+----+----+----+----+----+----+----+----+----+   22782


        G   E   V   V   F   P   L   S   I   N   R   S   A   L   R   R   A   E   F   V
        CCGGAGGTCCTGCCGGCCATGGTCAGGGCGGAAGCTGCGCCCGGCCAGGCCGAGGCG
22783   -----+----+----+----+----+----+----+----+----+----+----+----+   22842


        P   E   V   L   R   G   M   V   R   A   K   L   R   A   A   G   Q   A   E   A
        GCAGGGCCGAACGTGGTCGACCGGCTCGGTCGCCGGTCGGTCCCAGTCCCAGTCGCCGGG
22843   -----+----+----+----+----+----+----+----+----+----+----+----+   22902


        A   G   P   N   V   V   D   R   L   A   G   R   S   E   S   D   Q   V   A   G
        CTGGCCGAACTGGTCGTTCACACGCGGCGGGTCTCCGGGTACGGCTCGGCCGACCAG
22903   -----+----+----+----+----+----+----+----+----+----+----+----+   22962
```

FIG.2-60

```
          L  A  E  L  V  R  S  H  A  A  A  V  S  G  Y  G  S  A  D  Q
22963  CTCCCCGAGCGCAAGGCGTTCAAGGACCTCGGTTTCGACTCGCTGGCCGGCGGTGGAGCTG  23022
          L  P  E  R  K  A  F  K  D  L  G  F  D  S  L  A  A  V  E  L

          R  N  R  L  G  T  A  T  G  V  R  L  P  S  T  L  V  F  D  H
23023  CGCAACCGCCTCGGTACCGCGACCGGCGTGCGGGCTGCCCAGCACGTTGGTGTTCGACCAC  23082

          P  T  P  L  A  V  A  E  H  L  R  D  R  L  F  A  A  S  P  A
23083  CCGACTCCCGTGGGCGTGCCGAACACCTGCGGGACAGGCGTGTTCGGGCGCCTCACCGGCG  23142

          V  D  I  G  D  R  L  D  E  L  E  K  A  L  E  A  L  S  A  E
23143  GTGGGACATCGGCGACCGGCTGGACGAGCTGGAGAAGGCGGCTCGAAGCCCTGTCCGCCGAG  23202

          D  G  H  D  D  V  G  Q  R  L  E  S  L  L  R  R  W  N  S  R
23203  GACGGGCCACGACGACGTGGGCCAGCGCCTGGAGTCGCTGCGCCGGTGGAACAGCGCAGG  23262
       CGGGGCGGACGCCCCGAGCACGTCCGGCGATCAGCGAGGACGCGCCAGTGACGACGAGCTGTTC
```

FIG. 2-61

```
23263 ─────────┼─────────┼─────────┼─────────┼─────────┼─────────┼ 23322
          R   A   D   A   P   S   T   S   A   I   S   E   D   A   S   D   D   E   L   F

23323 ─────────┼─────────┼─────────┼─────────┼─────────┼─────────┼ 23382
      TCGATGCTCGACCAGCGGTTCGGCGGGGGAGGAGGACCTGTAGATGAGCGGTGACAACGGC
          S   M   L   D   Q   R   F   G   G   G   E   D   L   *   M   S   G   D   N   G

23383 ─────────┼─────────┼─────────┼─────────┼─────────┼─────────┼ 23442
      ATGACCGAGGAAAAGCTCCGGCGCTACCTCAAGCGCACCGTCACCGAGCTCGACTCGGTG
          M   T   E   E   K   L   R   R   Y   L   K   R   T   V   T   E   L   D   S   V

23443 ─────────┼─────────┼─────────┼─────────┼─────────┼─────────┼ 23502
      ACCGCGCGCCTGCGTGAAGTCGAGCACCGGGCCGGTGAGCCGATCGCCATCGTCGGCATG
          T   A   R   L   R   E   V   E   H   R   A   G   E   P   I   A   I   V   G   M

23503 ─────────┼─────────┼─────────┼─────────┼─────────┼─────────┼ 23562
      GCGTGCCGGTTCCCCGGCGACGTGGACTCGCCGGAGTCGTTCTGGGAGTTCGTGTCCGGC
          A   C   R   F   P   G   D   V   D   S   P   E   S   F   W   E   E   F   V   S   G

23563 ─────────┼─────────┼─────────┼─────────┼─────────┼─────────┼ 23622
      GGCGGGGACGCCATCGCGGAGGCCCCCGCCGACCGCGGCTGGGAGCCGGACCCCGACGCG
          G   G   D   A   I   A   E   A   P   A   D   R   G   W   E   P   D   P   D   A
```

FIG.2-62

```
23623                                                                                      23682
      CGGCTGGGCGGATGCTCCGGGCGCGGGCGACTTCGACGCGGGCTTCTTCGGGATCTCG
      -----+---------+---------+---------+---------+---------+--    -
      R   L   G   G   M   L   A   A   A   G   D   F   D   A   G   F   F   G   I   S

23683                                                                                      23742
      CCGCCGAGCGCGCTGGCGATGGACCCGCAGCAGCGGATCATGCTGGAGATCTCGTGGGAG
      -----+---------+---------+---------+---------+---------+--    -
      P   R   E   A   L   A   M   D   P   Q   Q   R   I   M   L   E   I   S   W   E

23743                                                                                      23802
      GCGCTGGAGCGCGGCCACGATCCGGTGTCCCTGCGCGGCAGCGCCGACCGGGGTGTTC
      -----+---------+---------+---------+---------+---------+--    -
      A   L   E   R   A   G   H   D   P   V   S   L   R   G   S   A   T   G   V   F

23803                                                                                      23862
      ACCGGTGTCGGCACCGTGGACTACGGCCCCGACCCGCGACCGAGGCCCCGACGAGGTCCTG
      -----+---------+---------+---------+---------+---------+--    -
      T   G   V   G   T   V   D   Y   G   P   R   P   D   E   A   P   D   E   V   L

23863                                                                                      23922
      GGCTACGTCGGCACCGCCTCCAGCGTCGGCCGCGTCGCCGGGTCGCCTACTGCCTG
      -----+---------+---------+---------+---------+---------+--    -
      G   Y   V   G   T   A   S   S   V   A   S   G   R   V   A   Y   C   L

23923                                                                                      23982
      GGCCTGGAAGGCCCGGCGATGACCGTCGACACCGCGTCTCCGGGCTCACCGCCCTG
      -----+---------+---------+---------+---------+---------+--    -
      G   L   E   G   P   A   M   T   V   D   T   A   C   S   S   G   L   T   A   L
```

```
23983  CACCTGGCGATGGAGTCGCTGCGCCGGGACGAGTGCGGCCTGGCGCTGGCCGGCGGCGTG  24042
       ------+---------+---------+---------+---------+---------+
       H  L  A  M  E  S  L  R  R  D  E  C  G  L  A  L  A  G  G  V

24043  ACGGTGATGAGCAGTCCCGGGGCGTTCACCGAGTTCCGCAGCCAGGGGCTCGCCGCC  24102
       ------+---------+---------+---------+---------+---------+
       T  V  M  S  S  P  G  A  F  T  E  F  R  S  Q  G  L  A  A

24103  GACGGCCGCTGCAAGCCGTTCTCGAAGGCCGCCGACGGGTTCGGCCTGGCCGAGGGTGCC  24162
       ------+---------+---------+---------+---------+---------+
       D  G  R  C  K  P  F  S  K  A  A  D  G  F  G  L  A  E  G  A

24163  GGGGTCCTGGTGCTGCAACGGCTGTCGCGCGGCCGGGAGGGCCAGACCGGTGCTGCGCC  24222
       ------+---------+---------+---------+---------+---------+
       G  V  L  Q  R  L  S  A  A  R  R  E  G  R  P  V  L  A

24223  GTGCTGCGGGGCTCGGCGGTCAACCAGGACGGCGCCAGCAACGGGCTGACCGCGCCAGC  24282
       ------+---------+---------+---------+---------+---------+
       V  L  R  G  S  A  V  N  Q  D  G  A  S  N  G  L  T  A  P  S

24283  GGACCCGGCGCAGCAGGCGGGCATCCGCCCGGCCGTGGAGAACGCCGGTGTCCGGGCGGGC  24342
       ------+---------+---------+---------+---------+---------+
```

FIG. 2-63

FIG.2-64

```
          G   P   A   Q   Q   R   V   I   R   R   A   L   E   N   A   G   V   R   A   G
24343  GACGTCGACTACGTGGAGGCCCACGGCACCGGCACCAGGCTGGGCGACCCCATCGAGGTG  24402
       -----+----+----+----+----+----+----+----+----+----+----+-----

          D   V   D   Y   V   E   A   H   G   T   G   T   R   L   G   D   P   I   E   V
24403  CACGCGCTGCTCTCGACCTACGGCGCCGGAACGGACACCCGGACGATCCACTGTGGATCGGT  24462
       -----+----+----+----+----+----+----+----+----+----+----+-----

          H   A   L   L   S   T   Y   G   A   E   R   D   P   D   D   P   L   W   I   G
24463  TCGGTCAAGTCCAACATTGGCCACACCCAGGCCGCCGCGGCGTCCCGGGGTGATGAAG  24522
       -----+----+----+----+----+----+----+----+----+----+----+-----

          S   V   K   S   N   I   G   H   T   Q   A   A   A   G   V   A   G   V   M   K
24523  GCGGGTGCTGGCGCTGGCACGGCGAGATGCCGGCACGCTGCACTTCGACGAGCCCTCG  24582
       -----+----+----+----+----+----+----+----+----+----+----+-----

          A   V   L   A   L   R   H   G   E   M   P   R   T   L   H   F   D   E   P   S
24583  CGCCAGATCCAGTGGGACCTGGGCGTGTCGGTGGTCGGAGGCGCGGTCGTGGCCC  24642
       -----+----+----+----+----+----+----+----+----+----+----+-----

          P   Q   I   E   W   D   L   G   A   V   S   V   V   S   Q   A   R   S   W   P
24643  GCCGGCGGAGAGGCCCCCAGGGGCGGCCGTCTCCTCGTTCGGCATCAGCGGCACCAACGCG  24702
       -----+----+----+----+----+----+----+----+----+----+----+-----
```

FIG.2-65

```
        A   G   E   R   P   R   R   A   G   V   S   S   F   G   I   S   G   T   N   A
      CACGTCATCGTCGAAGAGGCGGCCCGAGGCCGACGAGCCCGAGCCGGCACCCGACTCGGGT
24703 ----+----+----+----+----+----+----+----+----+----+----+----+ 24762

        H   I   V   E   E   A   P   E   A   D   E   P   E   A   P   P   D   S   G
      CCGGTCCCGCTGGTCGTTGTCCGGCCGGGCGACGAGCAGGCGCCGATGCGGGCCAGGCGGGACGG
24763 ----+----+----+----+----+----+----+----+----+----+----+----+ 24822

        P   V   P   L   V   L   S   G   R   D   E   Q   A   M   R   A   Q   A   G   R
      CTGGCAGACACCCTCGCCCCGCGAGCCGCGGAACTCGTTGCGCGACACCGGTtCTCACGCTG
24823 ----+----+----+----+----+----+----+----+----+----+----+----+ 24882

        L   A   D   H   L   A   R   E   P   R   N   S   L   R   D   T   G   F   T   L
      GCCACCCCGCCCAGCCGGTGGAGCACCGCGGTGGTGTCGGCGACCGCGACGACGCC
24883 ----+----+----+----+----+----+----+----+----+----+----+----+ 24942

        A   T   R   R   S   A   W   E   H   R   A   V   V   V   G   D   R   D   D   A
      CTCGCCGGGCTGCGACGCGTGGCCGACGGCCGCATCGCCGACGGCGACCGCCACCGGGCAG
24943 ----+----+----+----+----+----+----+----+----+----+----+----+ 25002

        L   A   G   L   R   A   V   A   D   G   R   I   A   D   R   T   A   T   G   Q
      GCCCGAACTCGCCGCGCGGCGTCGCGAtGGTGTTCCCCGGCCAGGGGCGGCGCAGTGGCAGGGG
25003 ----+----+----+----+----+----+----+----+----+----+----+----+ 25062
```

FIG. 2-66

```
            A  R  T  R  R  G  V  A  M  V  F  P  G  Q  G  A  Q  W  Q  G
25063 ATGGCCCGCGACCTGCTGCGGGAGTCGCAGGTATTCGCCGACTCGATCCGCGACTGGCAG  25122
      ------+---------+---------+---------+---------+---------+
      M  A  R  D  L  L  R  E  S  Q  V  F  A  D  S  I  R  D  C  E

            R  A  L  A  P  H  V  D  W  S  L  T  D  L  L  S  G  A  R  P
25123 CGGGCGCTGGCCCCGCACGTCGACTGGTCGCTGACCGACCTGCTCAGCGGCGCGCGACCCG  25182
      ------+---------+---------+---------+---------+---------+

            L  D  R  V  D  V  V  Q  P  A  L  F  A  V  M  V  S  L  A  A
25183 CTGGACCGGGTCGACGTCGTCCAGCCCGCCCTCTTCGCCGTCATGGTGTCGCTGGCGGCG  25242
      ------+---------+---------+---------+---------+---------+

            L  W  R  S  H  G  V  E  P  A  A  V  V  G  H  S  Q  G  E  I
25243 CTGTGGCGCTCCCACGGCGTCGAGCCCGCCGCGGTCGTCGGCCACTCGCAGGGCGAGATC  25302
      ------+---------+---------+---------+---------+---------+

            A  A  A  H  V  A  G  A  L  T  L  E  D  A  A  K  L  V  A  V
25303 GCCGCGGCGCACGTCGCCGGCGCGCTCACCCTGGAGGACGCCGCCAAGCTCGTCGCGGTC  25362
      ------+---------+---------+---------+---------+---------+
```

FIG.2-67

```
25363
      R   S   R   V   L   A   R   L   G   G   Q   G   G   M   A   S   F   G   L   G
      CGGAGCCGGGTCCTGGCCCGGCTCGGCGGGCGGCAGGGCGGCATGGGGTCGTTCGGGCTGGGC
      ---------+---------+---------+---------+---------+---------+--   25422

25423
      T   E   Q   A   A   E   R   I   G   R   F   A   G   A   L   S   I   A   S   V
      ACCGAGCAGGCGGCGGAACGGATCGGGCGGTTCGCGGGCGCCCTCTCCATCGCCTCGGTC
      ---------+---------+---------+---------+---------+---------+--   25482

25483
      N   G   P   R   S   V   V   V   A   G   E   S   G   P   L   D   E   L   I   A
      AACGGCCCCCGGTCGGTCGTCGTGGCCGGGGAGAGCGGGCCGCTGGACGAGCTGATCGCC
      ---------+---------+---------+---------+---------+---------+--   25542

25543
      E   C   E   A   E   G   I   T   A   R   R   I   P   V   D   Y   A   S   H   S
      GAGTGCGAGGCCGAAGGCCATAACGGCCGCCGCATCCCCGTCGACTACGCCTCCCACTCA
      ---------+---------+---------+---------+---------+---------+--   25602

25603
      P   Q   V   E   S   L   R   E   E   L   L   T   E   L   A   G   I   S   P   V
      CCGCAGGTGGAGTCCCTGCGCGAGGAGCTGCTGACCGAGCTGGCGGGCATCTCCCCGGTG
      ---------+---------+---------+---------+---------+---------+--   25662

25663
      TCGGGCGACGTGGCCGCTCTACTCGACCACGACGGGGCAGCCCCATCGACACCCGCCAGATG
      ---------+---------+---------+---------+---------+---------+--   25722
```

S   A   D   V   A   L   Y   S   T   T   T   G   Q   P   I   D   T   A   T   M

25723 GACACCGGCTACTGGTACCGGAACCTGCGGGAGCAGGTCCGCTTCCAGGACGCGACGCGCGG 25782
      ---------+---------+---------+---------+---------+---------+

D   T   A   Y   W   Y   A   N   L   R   E   Q   V   R   F   Q   D   A   T   R

25783 CAGCTCGCCGAGGCGGGGTTCGACGCGTTCGTCGAGGTCAGCCCGCATCCGGTGCTGACC 25842
      ---------+---------+---------+---------+---------+---------+

Q   L   A   E   A   G   F   D   A   F   V   E   V   S   P   H   P   V   L   T

25843 GTCGGCATCGAGGCCACGCTGGACTCCGCGCTCCCGGCGGACGCGGGCCCTGCGTCGTG 25902
      ---------+---------+---------+---------+---------+---------+

V   G   I   E   A   T   L   D   S   A   L   P   A   D   A   G   A   C   V   V

25903 GGCACCCTGCGGCGGGACCGCGGGCGGCCTGGCCGACTTCCACACCGCCTCCGGCGAGGCG 25962
      ---------+---------+---------+---------+---------+---------+

G   T   L   R   R   D   R   G   G   L   A   D   F   H   T   A   L   G   E   A

25963 TACGCGCAGGGCGTGGAGGTCGACTGGAGCCCCGCCTTCGCCGACGCGGCCGGTCGCAG 26022
      ---------+---------+---------+---------+---------+---------+

Y   A   Q   G   V   E   V   D   W   S   P   A   F   A   D   A   R   P   V   E

26023 CTGCCCGTCTACCCGTTCCAGGCGCAGCGGTACTGGCCTGCCCATCCCACCCGGCGGGGCGC 26082
      ---------+---------+---------+---------+---------+---------+

FIG.2-68

SUBSTITUTE SHEET

FIG.2-69

```
           L   P   V   Y   P   F   Q   R   Q   R   Y   W   L   P   I   P   T   G   G   R
26083  GCACGGGACGAGGACGACGACTGGCGCTACCAGGTCGTATGGCGGGAAGCCGAGTGGGAG
       -----+---------+---------+---------+---------+---------+  26142

           A   R   D   E   D   D   D   W   R   Y   Q   V   V   W   R   E   A   E   W   E
26143  AGCGCTTCCCTGGCCGGAGACGCGTCTCGTGGTGACCGGGCCGTGCCGTCCGAGTTG
       -----+---------+---------+---------+---------+---------+  26202

           S   A   S   L   A   G   R   V   L   L   V   T   G   P   G   V   P   S   E   L
26203  TCGGACGCCATCCGAAGTGACTGGCGCAGAGCGGTGCGACGGTCCTGACCTGCGACGTG
       -----+---------+---------+---------+---------+---------+  26262

           S   D   A   I   R   S   G   L   E   Q   S   G   A   T   V   L   T   C   D   V
26263  GAATCCCGTTCGACCATCGGCCACCGACTGGAGGCCGCCGACACCGACGCTCTGTCCACT
       -----+---------+---------+---------+---------+---------+  26322

           E   S   R   S   T   I   G   T   A   L   E   A   A   D   T   D   A   L   S   T
26323  GTGGTGTCGCTGCTGTCCCGCGACGGCGAGGCCGTCGATCCGTCGCTGGACGCGCTCGCC
       -----+---------+---------+---------+---------+---------+  26382

           V   V   S   L   L   S   R   D   G   E   A   V   D   P   S   L   D   A   L   A
26383  CTGGTCCAGGCCCTCGGAGCGCCGGGGTCGAAGCACCGCGTCTGGGTGCTGACCCGCAAC
       -----+---------+---------+---------+---------+---------+  26442
```

FIG.2-70

```
     L V Q A L G A A G V E A P L W V L T R N
      GCCGTGCAGGTGGCCGCGACGGCGAACTGGTTCGATCCGGCGCAGGCCATGGTGGCGGTCTC
26443 ------+---------+---------+---------+---------+---------+ 26502

     A V Q V A D G E L V D P A Q A M V G G L
      GGCCGGCTGGTCGGCATCGAGCACGCGGGGGCCGCTGGGGCGCGGTCGGTTGGTGACCTGGTCGAC
26503 ------+---------+---------+---------+---------+---------+ 26562

     G R V V G I E Q P G G R W G G L V D L V D
      GCCGATCGCCGCGTCGATCCGTCGCTGGCCGCGGTGCTGGCCGACCCGCGCGGCGAGGAG
26563 ------+---------+---------+---------+---------+---------+ 26622

     A D A A S I R S L A A V L A D P R G E E
      CAGGTCGCGATCGGCGGACGGGATCAAGGTGGCGAGGCTCGTGCCCCCGCCCGCCGC
26623 ------+---------+---------+---------+---------+---------+ 26682

     Q V A I R A D G I K V A R L V P A P A R
      GCCCACGCACCCCGTGGAGCCCTGCGACCGTGCTGTCACCGGCGCACCGGAGGG
26683 ------+---------+---------+---------+---------+---------+ 26742

     A A R T R W S P R G T V L V T G G T G G
      ATCGGCGCGCACGTGCCCCGCTGGCTGGCCCGCGCCGAGCACCTGGTGCTGCTG
```

FIG.2-71

26743

I G A H V A R W L A R S G A E H L V L L

GGCAGGCGCGGTGCCGACGCACCGGCGGCCGTCCGAGCTGGAGGAGCTGACCGCGCTC 26802

26803

G R R G A D A P G A S E L R E E L T A L

GGCACGGGCGGTGACCATCGCGCCGCCTGCGACGTCGCCGACGGGCGGCTCGAAGCGGTG 26862

26863

G T G V T I A A C D V A D R A R L E A V

CTCGCCGGCGGAGGCGCGCCGAGGGACCGCACCGTCAGCGCCGTGATGCACGCGCCGGGGGTT 26922

26923

L A A E R A E G R T V S A V M H A A G V

TCCACGTCCAGCGCCCCTCGACGACCTCACCGGAAGCCGAGTTCACCGAGATCGCCGACGTG 26982

26983

S T S T P L D D L T E A E F T E I A D V

AAGGTGCGGCGCCGCCACCGTCAACCTGGACGAGCTCTGCCCGGACCTCGACGCGTTCGTGTTG 27042

27043

K V R G T V N L D E L C P D L D A F V L 27102

FIG.2-72

```
27103                                                                              27162
      TTCTCCTCCAACGCGGGCGTGTGGGCAGTCCGGGGCTCGCCCTCCTACGCGGCGGCCAAC
      ---------+---------+---------+---------+---------+---------+
      F   S   S   N   A   G   V   W   G   S   P   G   L   A   S   Y   A   A   A   N

27163                                                                              27222
      GCCTTCCTCGACGCGGCTTCGCGCGGCGGCCGAGCGGCCGAGGGGCGCCGGCCGGTGACGTCCATC
      ---------+---------+---------+---------+---------+---------+
      A   F   L   D   G   F   A   R   R   R   S   E   G   A   P   V   T   S   I

27223                                                                              27282
      GCCTGGGGGCTCTGGGCCGGGCAGAACATGGCCGGGACGAGGGCGGCCGAGTACCTGCGC
      ---------+---------+---------+---------+---------+---------+
      A   W   G   L   W   A   G   Q   N   M   A   G   D   E   G   G   E   Y   L   R

27283                                                                              27342
      AGCCAGGGCCTCGCGGGCCATGGACCCGGATCGGGCCGTCGAGGAACTGCACATCACCCTC
      ---------+---------+---------+---------+---------+---------+
      S   Q   G   L   R   A   M   D   P   D   R   A   V   E   E   L   H   I   T   L

27343                                                                              27402
      GACCACGGTCAGACGTCCGTCGGTCGTGACATGGATCGCAGGCGCGGTTCGTCGTCGAGCTG
      ---------+---------+---------+---------+---------+---------+
      D   H   G   Q   T   S   V   V   D   M   D   R   R   R   F   V   E   L

27403                                                                              27462
      TTCACCGCGGCCCGGCACCCGGCCGCTGTTCCGACGAGATCGCCGGGCGTGCCCGGGCGGAAGCC
      ---------+---------+---------+---------+---------+---------+
      F   T   A   A   R   H   R   P   L   F   D   E   I   A   G   A   R   A   E   A
```

FIG.2-73

```
27463 CGGCAGAGCGAGGAGGGCCCGGCGCTCGCCCAGCGGCTCGCCGCGCTGTCGACGGCCGAG 27522
      ------+---------+---------+---------+---------+---------+
        R   Q   S   E   E   G   P   A   L   A   Q   R   L   A   A   L   S   T   A   E

27523 AGGCGCGAGCACCTCGCCCACCTGATCCGCGCCGAGTCGCCGCGGTGCTCGGCCACGGC 27582
      ------+---------+---------+---------+---------+---------+
        R   R   E   H   L   A   H   L   I   R   A   E   V   A   A   V   L   G   H   G

27583 GACGACGGCGGCGATCGACCGCGACGCCTTCCGGGACCTCGGCTTCGACTCCATGACC 27642
      ------+---------+---------+---------+---------+---------+
        D   D   A   A   I   D   R   D   R   A   F   R   D   L   G   F   D   S   M   T

27643 GCCGTCGACCTGCGGAACCGGCTCGCCGCGGTGACCGGGGTGCGGGAGGCCGCGACCGTG 27702
      ------+---------+---------+---------+---------+---------+
        A   V   D   L   R   N   R   L   A   A   V   T   G   V   R   E   A   A   T   V

27703 GTCTTCGACCACCCGACCATCACCCGGCTCGCCGACCACTACCTGGAGCGGCTCGTCGGC 27762
      ------+---------+---------+---------+---------+---------+
        V   F   D   H   P   T   I   T   R   L   A   D   H   Y   L   E   R   L   V   G

27763 GCAGCAGAGGCGGGAGCAAGCCCCGCGCTCGTGCGCGAGGTGCCGAAGGATGCCGACGAC 27822
      ------+---------+---------+---------+---------+---------+
        A   A   E   E   Q   A   P   A   L   V   R   E   V   P   K   D   A   D   D
```

FIG.2-74

```
27823 CCGATCGCGATCGTCGGCATGGCCTGCCGCTTCCCCGGCGGGCGTGCACAACCCGGTGAG 27882
      ---------+---------+---------+---------+---------+---------+
       P   I   A   I   V   G   M   A   C   R   F   P   G   G   V   H   N   P   G   E   -

27883 CTGTGGGAGTTCATCGTCGGCCGCGGAGACGCCGTGACGGAGATGCCCACCGACCGCGGC 27942
      ---------+---------+---------+---------+---------+---------+
       L   W   E   F   I   V   G   R   G   D   A   V   T   E   M   P   T   D   R   G   -

27943 TGGGACCTCGACGGCGTGTTCGACCCCGACCCGCAGCCCACGGAACCAGCTACTCGGCGA 28002
      ---------+---------+---------+---------+---------+---------+
       W   D   L   D   A   L   F   D   P   D   P   Q   R   H   G   T   S   Y   S   R   -

28003 CACGGCGCGTTCCTCGACGGGCCGGCCGACTTCGACGCGCGGTTCTTCGGGATCTCGCCG 28062
      ---------+---------+---------+---------+---------+---------+
       H   G   A   F   L   D   G   A   A   D   F   D   A   A   F   F   G   I   S   P   -

28063 CGCGAGGCGCTGGCGATGGACCCGCAGCAGCGCCAGGTCCTGGAAACGACGTGGGAGCTG 28122
      ---------+---------+---------+---------+---------+---------+
       R   E   A   L   A   M   D   P   Q   Q   R   Q   V   L   E   T   T   W   E   L   -

28123 TTCGAGAACGCCCGCATCGACCCGCACTCGCTGCGGGGCAGCGACACCGGCGTCTTCCTC 28182
      ---------+---------+---------+---------+---------+---------+
```

FIG.2-75

```
        F   E   N   A   G   I   D   P   H   S   L   R   G   S   D   T   G   V   F   L
28183  GGCGCCGCTACCAGGCTACGGCCAGGACACGGCGGTGGTGCCCGAGGACACGCGAGGGCTAC  28242
       --------+---------+---------+---------+---------+---------+--

        G   A   A   Y   Q   G   Y   G   Q   D   A   V   V   P   E   D   S   E   G   Y
28243  CTGCTCACCGGCAACTCCGCCGTGGTGTCCGGCCGGGTCGCCTACGTGCTGGGGCTG  28302
       --------+---------+---------+---------+---------+---------+--

        L   L   T   G   N   S   A   V   V   S   G   R   V   A   Y   V   L   G   L
28303  GAAGGCCCCGCCGGGGTCACGGTGGACACGGCGTGTTCGTCGTCGTTGGTGGCCTTGCATTCG  28362
       --------+---------+---------+---------+---------+---------+--

        E   G   P   A   V   T   V   D   T   A   C   S   S   S   L   V   A   L   H   S
28363  GCGTGTGGGTCGTTGCGTGACGGTGACTGCGGGTCTTGCGGTGGCCCGTGGTGTCGGTG  28422
       --------+---------+---------+---------+---------+---------+--

        A   C   G   S   L   R   D   G   D   C   G   L   A   V   A   G   G   V   S   V
28423  ATGGCCGGCCCCGGAGTGTTCACCGAGTTCTCCCGCCAGGGCGGCTTGGCCCGTGGACGGG  28482
       --------+---------+---------+---------+---------+---------+--

        M   A   G   P   E   V   F   T   E   F   S   R   Q   G   G   L   A   V   D   G
28483  CGCTGCAAGGCGTTCTCCGCGGAGGCCGACACGGCGCTTCGGTTTCGCGCAGGGCCTCGCCGGTG  28542
       --------+---------+---------+---------+---------+---------+--
```

FIG.2-76

```
          R   C   K   A   F   S   A   E   A   D   G   F   G   F   A   E   G   V   A   V
       GTCCTGCTCCAGCCGGTTGTCCGACGCCCGCAGGGCGGGTCGCCAGTGCTGCTGCGGTGTC
28543  -----+---------+---------+---------+---------+---------+---------+  28602

          V   L   L   Q   R   L   S   D   A   R   R   A   G   R   Q   V   L   G   V   V
       GCGGGCTCGGCGATCAACCAGGACGGGCGCGAGCAACGGTCTCGCGGCGCCCAGCCGCGTC
28603  -----+---------+---------+---------+---------+---------+---------+  28662

          A   G   S   A   I   N   Q   D   G   A   S   N   G   L   A   A   P   S   G   V
       GCCCAGCAGCGCGTGATCCGCAAGGCGTGGGCGCGTGCGGGGATCACCGGCGCGGATGTG
28663  -----+---------+---------+---------+---------+---------+---------+  28722

          A   Q   Q   R   V   I   R   K   A   W   A   R   A   G   I   T   G   A   D   V
       GCCGTGGTGGAGGCCCATGGGACCGGCTGGCGCCTGGCGATCCGGTGGAGGCGTCGGCG
28723  -----+---------+---------+---------+---------+---------+---------+  28782

          A   V   V   E   A   H   G   T   G   T   R   L   G   D   P   V   E   A   S   A
       TTGCTGGCTACTTACGGCAAGTCGCGCGGGTCGTCGGGCCCCGTGCTGCTGGGTTCGGTG
28783  -----+---------+---------+---------+---------+---------+---------+  28842

          L   L   A   T   Y   G   K   S   R   G   S   S   G   P   V   L   L   G   S   V
```

FIG.2—77

```
28843
        K  S  N  I  G  H  A  Q  A  A  A  G  V  A  G  V  I  K  V  V
    AAGTCGAACATCGTCACGCGCAGGCGGCCCGGGTGTCGCGGCGGCTGATCAAGGTGGTC
    ---+----+----+----+----+----+----+----+----+----+----+----+
                                                            28902

28903
        L  G  L  N  R  G  L  V  P  P  M  L  C  R  G  E  R  S  P  L
    CTGGGGTTGAACCGCGGCCTGGTGCCGCCGATGCTCTGCCGCGGCGAGCGGTCGCCGCTG
    ---+----+----+----+----+----+----+----+----+----+----+----+
                                                            28962

28963
        I  E  W  S  S  G  G  V  E  L  A  E  A  V  S  P  W  P  P  A
    ATCGAATGGTCCTCGGGTGGTGTGGAACTTGCCGAGGCCCGTGAGCCCGTGGCCTCCGGCC
    ---+----+----+----+----+----+----+----+----+----+----+----+
                                                            29022

29023
        A  D  G  V  R  R  A  G  V  S  A  F  G  V  S  G  T  N  A  H
    GCGGACGGGGTGCCGCGGGCCGGTGTGTCGGCGTTCGGGGTGAGCGGGACGAACGCGCAC
    ---+----+----+----+----+----+----+----+----+----+----+----+
                                                            29082

29083
        V  I  A  E  P  P  E  P  L  P  E  P  G  P  V  G
    GTGATCATCGCCGAGCCCCGGAGCCGCTGCCGGAACCCGACCGGTGGCCGTGGGCCGTG
    ---+----+----+----+----+----+----+----+----+----+----+----+
                                                            29142

29143
    CTGGCCGCTGCGAACTCGGTGCCCGTACTGCTGTCGCCCAGGACCGAGACCGCGGTTGGCA
    ---+----+----+----+----+----+----+----+----+----+----+----+
                                                            29202
```

```
          L   A   A   A   N   S   V   P   V   L   L   S   A   R   T   E   T   A   L   A
29203 GCGCAGGCGGCGGCTCCTGGAGTCCGCAGTGGACGACTCGGTTCCGTTGACGGCATTGGCT
      ------+---------+---------+---------+---------+---------+  29262
```

```
          A   Q   A   R   L   L   E   S   A   V   D   D   S   V   P   L   T   A   L   A
29263 TCCGCGCTGGCCACCGGACGCGCCCACCTGCCGCGTCGTGGCGGCGTTGCTGGCAGGCGAC
      ------+---------+---------+---------+---------+---------+  29322
```

```
          S   A   L   A   T   G   R   A   H   L   P   R   R   A   A   L   L   A   G   D
29323 CACGAACAGCTCCGCGGCCAGTTGCGAGCGGTCGCCGAGGGCGTTGCGGCTCCCGGTGCC
      ------+---------+---------+---------+---------+---------+  29382
```

```
          H   E   Q   L   R   G   Q   L   R   A   V   A   E   G   V   A   A   P   G   A
29383 ACCACCGGAACCGCCTCCGCGGCGGGCGTGGTTTCGTCTTCCCAGTCAGGGTGCTCAG
      ------+---------+---------+---------+---------+---------+  29442
```

```
          T   T   G   T   A   S   A   G   G   V   V   F   V   F   P   G   Q   G   A   Q
29443 TGGGAGGGCATGGCCCGGGGCTTGCTCTCGGTCCCGTCTTCGCGAGTCGATCGCCGAG
      ------+---------+---------+---------+---------+---------+  29502
```

```
          W   E   G   M   A   R   G   L   L   S   V   P   V   F   A   E   S   I   A   E
29503 TGCGATCGCGGTGTTGTCGGAGGTGGCCGGGGTTCTCGGCCTCCGAAGTGCTGGACCAGCGT
      ------+---------+---------+---------+---------+---------+  29562
```

FIG.2-78

FIG.2-79

```
29563
         C   D   A   V   L   S   E   V   A   G   F   S   A   S   E   V   L   E   Q   R
         CCGGACGCGCCCGTCGCTGCTCGAGCCGGTCGCGACGTCGTACAGCCGGTCGTTGTTCTCCGTGATG
         ----+----+----+----+----+----+----+----+----+----+----+----+----+
                                                                        29622

29623
         P   D   A   P   S   L   E   R   V   D   V   V   Q   P   V   L   F   S   V   M
         GTGTCGCTGGCGCCGCTGTGGGGCGCTGCCGGAGTCAGCCCCTCCGCCGTCATCGGCCAT
         ----+----+----+----+----+----+----+----+----+----+----+----+
                                                                        29682

29683
         V   S   L   A   R   L   W   G   A   C   G   G   V   S   P   S   A   V   I   G   H
         TCGCAGGGCGAGATCGCCGCCGCGGTGGTGGCCGGGGTGTTGTCGCTGGAGGACGGCGTG
         ----+----+----+----+----+----+----+----+----+----+----+----+
                                                                        29742

29743
         S   Q   G   E   I   A   A   A   V   V   A   G   V   L   S   L   E   D   G   V
         CGCGTCGTGGCCCTGCGCGCCAAGGCGTTGCGTGCGCGCCTGGCCGGGGCCAAGGGCGGCCATGGTC
         ----+----+----+----+----+----+----+----+----+----+----+----+----+
                                                                        29802

29803
         R   V   V   A   L   R   A   K   A   L   R   A   L   A   G   K   G   G   M   V
         TCGTTGGCGGCTCCCGGTGAACGGCGCCGCCGCGCTGATCGCACCGTGGGAGGACCGGATC
         ----+----+----+----+----+----+----+----+----+----+----+----+
                                                                        29862

29863
         S   L   A   A   P   G   E   R   A   R   A   L   I   A   P   W   E   D   R   I
         TCCGTCGCGGGCGGTCAACTCCGTCGTCGGTCTCCGGCGATCCGGCGAGGCGGCTG
         ----+----+----+----+----+----+----+----+----+----+----+----+
                                                                        29922
```

```
     S   V  A  A  V  N  S  P  S  S  V  V  V  S  G  D  P  E  A  L
29923 GCCGAACTCGTCGCACGTTGCGAGGACGAGGGCCGTGCGCGCCAAGACGCTCCCGGTGGAC 29982
      -----+---------+---------+---------+---------+---------+----

     A   E  L  V  A  R  C  E  D  E  G  V  R  A  K  T  L  P  V  D
29983 TACGCCTCGACTCCCGCCACGTCGAGGAGATCGCGGAGACGATCCTCGCCCGACCTCGAC 30042
      -----+---------+---------+---------+---------+---------+----

     Y   A  S  H  S  R  H  V  E  E  I  R  E  T  I  L  A  D  L  D
30043 GGCATCTCCGCGGGCGGCGTGCCGCCATCCCGCTCTACTCCACGTGCACGGCGGAACGGCGC 30102
      -----+---------+---------+---------+---------+---------+----

     G   I  S  A  R  R  A  A  I  P  L  Y  S  T  L  H  G  E  R  R
30103 GACGGGCGCCGACATGGGTCCGGCGTACTGGTACGACAACCTGCGCTCCCAGGTGCGCTTC 30162
      -----+---------+---------+---------+---------+---------+----

     D   G  A  D  M  G  P  R  Y  W  D  N  L  R  S  Q  V  R  F
30163 GACGAGGCGGTCTCGGCCGCGTCGCCGACGGTCACGGCACCTTCGTCGAGATGAGCCCG 30222
      -----+---------+---------+---------+---------+---------+----

     D   E  A  V  S  A  A  V  A  D  G  H  A  T  F  V  E  M  S  P
      CACCCGGTGCTCACCGGCGGGGTGCAGGAGATCGCCGGGACCGCGGACCGCCATCGGGTCG
```

FIG.2—80

FIG. 2-81

```
30223 --------------------------------------------------------- 30282
        H   P   V   L   T   A   A   V   Q   E   I   A   A   D   A   V   A   I   G   S
      CTGCACCGGCGACACCGGGAGGAGCACCTGATCGCCGCCGAGCTCGCCCGGGCGCACGTGCAC
30283 ---------+---------+---------+---------+---------+---------+--- 30342
        L   H   R   D   T   A   E   E   H   L   I   A   E   L   A   R   A   H   V   H
      GGCGTGGCCGGTGGACTGGCGGAACGTCTTCCCGGCGCGCCACCTCCGGTGGCGCTGCCCAAC
30343 ---------+---------+---------+---------+---------+---------+--- 30402
        G   V   A   V   D   W   R   N   V   F   P   A   A   P   P   V   A   L   P   N
      TACCCGTTCGAGCCCCAGCGGTACTGGCTCGCGCCCGGAGGTGTCCGACCAGCTCGCCGAC
30403 ---------+---------+---------+---------+---------+---------+--- 30462
        Y   P   F   E   E   P   Q   R   Y   W   L   A   P   E   V   S   D   D   Q   L   A   D
      AGCCGCTACCGGCGTCGACTGGCCACCCTGGCCACCGCCGGTCGGACCTGGAAGGCGGGC
30463 ---------+---------+---------+---------+---------+---------+--- 30522
        S   R   Y   R   V   D   W   R   P   L   A   T   T   P   V   D   L   E   G   G
      TTCCTGTGGTCCACCGGGTCCGCACCGGAGTCGCTGACCAGCCGCAGTCGAGAAGGCCGGGAGGC
30523 ---------+---------+---------+---------+---------+---------+--- 30582
        F   L   V   H   G   S   A   P   E   S   L   T   S   A   V   E   K   A   G   G
```

FIG 2-82

```
30583 CGGCTCGTGCCGTCGCTCCGGCGACCGGCGAAGCCTCGGCGGCCTCGCGGGGAGGTGCCG 30642
      -----+----+----+----+----+----+----+----+----+----+----+
       R   V   V   P   V   A   S   A   D   R   E   A   S   A   A   L   R   E   V   P

30643 GGCGAGGTCGCCGGCGTGCTCTCGGTCGCTGGTCCACACCGGCGCCGCAACGCCACCTCGCCCTGCAC 30702
      -----+----+----+----+----+----+----+----+----+----+----+
       G   E   V   A   G   V   L   S   V   H   T   G   A   A   T   H   L   A   L   H

30703 CAGTCGCTGGGTGAGGCCGGCGTGCGGGCCCGCGTCTGCTGGTCACCAGCCAGCGGTC 30762
      -----+----+----+----+----+----+----+----+----+----+----+
       Q   S   L   G   E   A   G   V   R   A   P   L   W   L   V   T   S   R   A   V

30763 GCGCTCGGGGAGTCCGAGCCGGTCGATCCCGAGCAGCAGGCGATGGTGTGGGGTCTCGGGCGC 30822
      -----+----+----+----+----+----+----+----+----+----+----+
       A   L   G   E   S   E   P   V   D   P   E   Q   A   M   V   W   G   L   G   R

30823 GTCATGGGCCTGAGAGACCCCGGAACGGTGGGGCGGTTCGTCGCCTGCCTCGGCGGACGGACCGAACCC 30882
      -----+----+----+----+----+----+----+----+----+----+----+
       V   M   G   L   E   T   P   E   R   W   G   G   L   V   D   L   P   A   E   P

30883 GCGCCGGGGACGGCGAGGCGGTTCGTCGCCTGCCTCGGCGGACGGCACGAGGACCAG 30942
      -----+----+----+----+----+----+----+----+----+----+----+
       A   P   G   D   G   E   A   F   V   A   C   L   G   A   D   G   H   E   D   Q
```

```
30943 GTCGCGATCCGTGACCACGCCCGCTACGGCGCCGCCCTTCGTCCGCCGCCCGCTGGGCACC
      ------+---------+---------+---------+---------+---------+ 31002
       V  A  I  R  D  H  A  R  Y  G  R  R  L  V  R  A  P  L  G  T

31003 CGCGAGTCGAGCTGGGAGCCCGGGCACCGGCGCTGGTCACCGGCGGCACCGTGCCGCTC
      ------+---------+---------+---------+---------+---------+ 31062
       R  E  S  S  W  E  P  A  G  T  A  L  V  T  G  G  T  G  A  L

31063 GGCGGCCACGTCGCCCGCCACCTCGCCCAGTGCGGGGGTGGAGGACCTGGTGCTGGTCAGC
      ------+---------+---------+---------+---------+---------+ 31122
       G  G  H  V  A  R  H  L  A  R  C  G  V  E  D  L  V  L  V  S

31123 AGGCGCGGCGTCGACCGCTCCCGGCGCGGCCGAGCTGGAAGCCGAACTGGTCGCCCTCGGC
      ------+---------+---------+---------+---------+---------+ 31182
       R  R  G  V  D  A  P  G  A  A  E  L  E  A  E  L  V  A  L  G

31183 GCGAAGACGACCATCACCGCCTGCGACGTGGCCGACCGCGAGCAGCTCTCCAAGCTGCTG
      ------+---------+---------+---------+---------+---------+ 31242
       A  K  T  I  T  A  C  D  V  A  D  R  E  Q  L  S  K  L  L

31243 GAAGAACTGCGCGGGGCAGGGACGTCCGGTGCGGACCGTCGTGCACACCGCCGGGGTGCCC
      ------+---------+---------+---------+---------+---------+ 31302
```

FIG. 2-83

FIG.2-84

```
          E   E   L   R   G   Q   Q   G   R   P   V   R   T   V   V   H   T   A   G   V   P
31303 GAATCGAGGCCCTGACGAGATCGGCGAGCTGGAGTCGGTCTGCCGCCGCGAAGGTGACC 31362
      ------+---------+---------+---------+---------+---------+

          E   S   R   P   L   H   E   I   G   E   L   E   S   V   C   A   A   K   V   T
31363 GGGCCCCGCTGCTCGACGAGCTGTGCCCGGACGCGCCGAGACCTTCGTCCTGTTCTCGTCC 31422
      ------+---------+---------+---------+---------+---------+

          G   A   R   L   L   D   E   L   C   P   D   A   E   T   F   V   L   S   S
31423 GGAGCGGGGGTGTGGGGCAGTGCGAACCTCGGGCGGCCTACTCCGGGCCAACGCCTACCTC 31482
      ------+---------+---------+---------+---------+---------+

          G   A   G   V   W   G   S   A   N   L   G   A   Y   S   A   A   N   A   Y   L
31483 GACGCGCTGGCCCACCGCCGTGCGGAAGGCCGTGCCGGCGACGTCCGTCGCGTGGGGC 31542
      ------+---------+---------+---------+---------+---------+

          D   A   L   A   H   R   R   R   A   E   G   R   A   A   T   S   V   A   W   G
31543 GCCTGGGCGGGCCGAGGGCATGGCCACCGGCGACCTCGAGGGGCTCACCGGCGGCCTG 31602
      ------+---------+---------+---------+---------+---------+

          A   W   A   G   E   G   M   A   T   G   D   L   E   G   L   T   R   R   G   L
31603 CGCCCGATGGCCCCGAGCGGCGGGATCCGGCGCTGCACCAGGCCTGCACAACGGCGAC 31662
      ------+---------+---------+---------+---------+---------+
```

FIG. 2-85

```
      R  P  M  A  P  E  R  A  I  R  A  L  H  Q  A  L  D  N  G  D
      ACGTGCGTTCGATCGCCGACGTCGACTGGGAGGCCTTCGCGGTCGGCTTCACCGCCGCC
31663 ----+----+----+----+----+----+----+----+----+----+----+---- 31722

      T  C  V  S  I  A  D  V  D  W  E  A  F  A  V  G  F  T  A  A
      CGGCCCGCGTCCGCTGCTGACGAGCTCGTCACGCCGGCGGTGGGGGCCGGTCCCCGCGGTG
31723 ----+----+----+----+----+----+----+----+----+----+----+---- 31782

      R  P  R  P  L  L  D  E  L  V  T  P  A  V  G  A  V  P  A  V
      CAGGCGGCCCCGCCGCGGGAGGATGACGTCGCAGGAGTTGCTCGGAGTTCACGCACTCCCAC
31783 ----+----+----+----+----+----+----+----+----+----+----+---- 31842

      Q  A  A  P  A  R  E  M  T  S  Q  E  L  L  E  F  T  H  S  H
      GTCGGCGGCGATCCTCGGGCATTCCAGCCGGACCGGTCGGGCAGGACCAGCCGTTCACC
31843 ----+----+----+----+----+----+----+----+----+----+----+---- 31902

      V  A  A  I  L  G  H  S  S  P  D  A  V  G  Q  D  Q  P  F  T
      GAGCTCGGCTTCGACTCGCTGACGCGGGTGCGGCTGCGCAACCAGCTCCAGCAGGCCACC
31903 ----+----+----+----+----+----+----+----+----+----+----+---- 31962

      E  L  G  F  D  S  L  T  A  V  G  L  R  N  Q  L  Q  Q  A  T
      GGGCTCGGCGCTCCCCGACCCTGGTGTTCGAGCACCCCACGGTCGCCAGGTTGGCCCGAC
31963 ----+----+----+----+----+----+----+----+----+----+----+---- 32022
```

FIG.2-86

```
         G   L   A   L   P   A   T   L   V   F   E   H   P   T   V   R   R   L   A   D
         CACATAGGACACAGCTCGACAGCGGACTCCCCGCCCCGGGAAGCGAGCAGCGGCTCTTCGC
32023    ---------+---------+---------+---------+---------+---------+    32082

         H   I   G   Q   Q   L   D   S   G   T   P   A   R   E   A   S   A   L   R
         GACGGCTACCGGCAGCGGCGGGCCTGTCGGCAGGGTCCGGCTCCTACCTCGACCTGCTGGCG
32083    ---------+---------+---------+---------+---------+---------+    32142

         D   G   Y   R   Q   A   G   V   S   G   R   V   R   S   Y   L   D   L   L   A
         GGGCTGTCGGACTTCCGGCGAGCCACTTCGACGGCGTCCGACGGGTTCTCCCTCGATCTCGTG
32143    ---------+---------+---------+---------+---------+---------+    32202

         G   L   S   D   F   R   E   H   F   D   G   S   D   G   F   S   L   D   L   V
         GACATGGCCGACGGTCCCGGAGAGTCACCGTGATCTGCTGCGCGGGAACGGCGGCGATC
32203    ---------+---------+---------+---------+---------+---------+    32262

         D   M   A   D   G   P   G   E   V   T   V   I   C   C   A   G   T   A   A   I
         TCCGGTCCGCAGAGTTCACCCGGGCTCGCCGGGGCTGCGCGGAATCCTCCGGTTCGG
32263    ---------+---------+---------+---------+---------+---------+    32322

         S   G   P   H   E   F   T   R   L   A   G   A   L   R   G   I   A   P   V   R
```

FIG.3
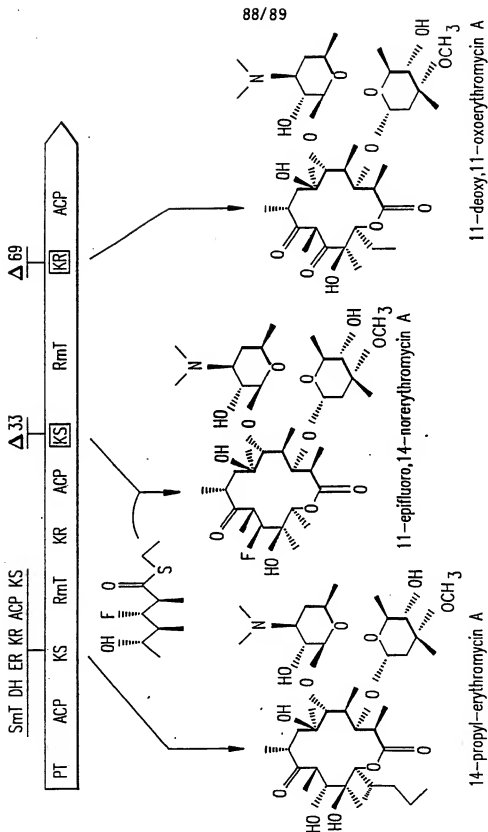
89/89

NUMBER	SITE	DISTANCE (Kb)[a]
1	BamHI	−3.60
2	PvuII	−3.50
3	PvuII	−3.40
4	PstI	−3.05
5	BamHI	−2.95
6	XhoI	−2.80
7	PstI	−2.00
8	HindII	−1.60
9	SphI	−1.55
10	EcoRI	−1.50
11	KpnI	−1.35
12	EcoRI	−1.05
13	SmaI [b]	−0.90
14	SphI	−0.75
15	KpnI	−0.65
16	SmaI	−0.20

FIG.4

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): A01N 43/22; A61K 31/71; C07H 17/08; C12N 1/21; C12P 19/62
US CL : 435/76, 252.35, 886; 514/29; 536/7.2

II. FIELDS SEARCHED

Minimum Documentation Searched[4]	
Classification System	Classification Symbols
U.S.	435/76, 183, 252.35, 886; 514/29; 536/7.2

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched[5]
APS, BIOSIS, MEDLINE, BIOTECH. ABSTRACTS

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category[*]	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
Y	J. of Bacteriology, Volume 164, No. 1, issued October 1985, J.M. Weber et al, "Genetic Analysis of Erythromycin Production in Streptomyces erythreus", pages 425-433, See the entire document.	1-30
Y	J. of Bacteriology, Volume 172, No. 5, issued May 1990, J.M. Weber et al, "Organization of a Cluster of Erythromycin Genes in Saccharomyces erythraea", pages 2372-2383, See the entire document.	1-30
Y	US, A, 4,874,748 (Katz et al) 17 October 1989, see column 1, lines 41-59; column 3, lines 47-60.	1-30
Y	US, A, 4,935,340 (Baltz et al) 19 June 1990, see entire document.	1-29

* Special categories of cited documents:[16]
"A" document defining the general state of the art which is not considered to be of particular relevance
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
20 MARCH 1992	3 0 MAR 1992
International Searching Authority[1]	Signature of Authorized Officer[20]
ISA/US	Dian Cook

Form PCT/ISA/210 (second sheet)(May 1986) &